# Status of Program

The Agricultural Research Services' (ARS) major research programs—New Products/Product Quality/Value Added; Livestock/Crop Production; Food Safety; Livestock/Crop Protection; Human Nutrition; and Environmental Stewardship—address the Department's goals and priorities. A brief summary of the agency's selected accomplishments and current research activities including the National Agricultural Library are detailed below.

NOTE: Those accomplishments with yellow highlighted national program coding at the end of the paragraph were the edited versions used in the budget explanatory notes

New Products/Product Quality/Value Added (ARS Goal 2) (306, 213)

**Select Examples of Recent Progress:** 

# **NP306**

New, rapid digital imaging wheat sorting system developed. Single grain wheat sorting is traditionally a human, hand-held visual detection of mold, weather, disease, and storage damaged grains. Although several attempts have been made over the decades to develop instrument-based alternatives, inspection still remains a challenge. ARS scientists from Beltsville, Maryland, have developed a digital imaging system that captures images of individual seeds in freefall. The imaging system, coupled with rapid image processing, scans more than 82 percent of each seed during high speed sorting. This new system will affect the inspection/grading of U.S. wheat and its trading and milling industries. It also is being tested by a pharmaceutical company for assessing the quality of pill coatings. Licensing and patent applications are being filed. (NP306, C 2, P.S. 2A, P.M. 2.1.2, Project # 1245-44000-009-00D)

Milk processing plant simulator lowers greenhouse gas emissions. Life cycle assessments of on-farm and off-farm fluid milk production found on-farm activities generated 70 percent of greenhouse gas emissions due to methane from cows and manure. Off-farm activities such as milk processing, packaging, and refrigeration contributed 30 percent. Significant reductions in greenhouse gas emissions for off-farm processors are practical only if processors know the energy hot spots in their plants and if the costly upgrades will reduce greenhouse gas emissions. ARS scientists in Wyndmoor, Pennsylvania, partnered with dairy processors to create a computer-based model of the fluid milk process to lower greenhouse gas emissions. The model can offer multiple ways of making changes in individual processing plants and instantly calculate both greenhouse gas reductions and costs of implementing the changes. The model has been distributed to more than 100 processors in the United States and should help the dairy industry realize its goal of reducing greenhouse gas emissions by 25 percent per gallon of milk by 2020. (NP 306, C I, P.S. 1C and 1D, P.M. 2.1.2, Project # 1935-41000-087-00D)

Novel nanoparticles improve glass window cleaners. While nanoparticles have attractive industrial properties, when used in liquids over time they bind together, fall out of solution, and become ineffective. ARS scientists in Peoria, Illinois, developed a biodegradable solution of protein nanoparticles that when applied to solid surfaces such as glass, spread out and prevent the beading of water. Near term application includes solar panels and side windows on vehicles that have no wipers. This technology is expected to show superiority over current, commercially available cleaners. An invention disclosure has been submitted and a patent application is in process. (NP 306, C2, P.S. 2B, P.M. 2.1.2, Project # 3620-44000-051-00D)

Packaging inserts that fights decay of fresh produce. Decay of fresh produce, especially small fruits such as strawberries and blueberries that cannot be washed, contributes to a short postharvest shelf life. ARS scientists in Fort Pierce, Florida, in collaboration with a CRADA partner, developed small, single use packets that when inserted into packaged fruit release an antimicrobial vapor (Curoxin) that surrounds the fresh fruit. The vapor extended the postharvest shelf life of blueberries and strawberries by maintaining fruit firmness, reducing water loss and decay, and maintaining color and overall quality. It was also used to treat citrus fruit infected with citrus canker. Canker is a problem for the fresh citrus market because fruit coming from groves where canker is found cannot be marketed internationally. Packets containing the vaporizing compound placed inside containers of citrus significantly reduced bacterial canker counts. The antimicrobial vapor packets are being tested in pilot studies with commercial packing

houses. The packets could save the international fresh produce industry more than \$1 billion annually. (NP 306, C1, P.S. 1B, P.M. 2.1.2, Project #6618-41430-005-00D)

New healthy functional foods from oats. Studies revealed that the soft-solid characteristics of various oat carbohydrates (beta-glucan) provided creamier, less runny properties that are valuable for developing new functional foods such as yogurt, instant puddings, custard, batter, smoothies, and ice cream. ARS scientists in Peoria, Illinois, developed the oat concentrates, which appear to have great potential for health concerned consumers. An industrial partner has licensed this ARS patented digestible, functional food from oats for the production of Calorie-Trim and Nutrim. Z-Trim is licensing this product for expanded markets, including USDA's school lunch program. (NP 306, C 1, P.S. 1C, P.M. 2.1.2, Project # 3620-41000-151-00D)

New, rapid, low-cost process for making nano materials. The promise of nanotechnology for use in manufacturing biobased packaging materials has been slowed by the inability to scale up the production of nano-based materials and be cost-effective. ARS scientists in Albany, California, have developed a new spinning process that blows nano-type materials onto surfaces that is much cheaper and can be more rapidly scaled up compared with the industry standard, electrospinning. The specific advantages of nano-scale blow-spinning include a higher fiber production rate; an ability to scale up production using inexpensive, commercially available components; an ability to blow nano-based materials onto surfaces without consideration of their electrical charge; its relative portability; and savings (i.e., no high-voltage equipment is required). A patent has been filed. Currently, blow-spinning using bio-based materials is being tested by a large convenience food company to reduce noisy packages. (NP 306, C 2 and C 3, P.S. 2A, 3a and 3B, P.M. 2.1.2, Project # 5325-41000-056-00D)

White grape seed flour may lower cholesterol and weight gain. Grape seeds, a waste byproduct of the wine-making process, are a disposal issue for producers. ARS scientists in Albany, California, found that flour made from chardonnay grape seeds, but not red grapes or grape skins, prevented increases in plasma cholesterol and weight gain in hamsters fed a high-fat diet. In subsequent research, the scientists measured changes in the metabolic pathways of cholesterol and fat metabolism that supported the weight-loss observations. The sale of grape seeds for milling into flour has the potential to offer an additional revenue stream for wine producers. An invention disclosure has been submitted, and human clinical trials are underway at the Mayo Clinic. (NP 306, C1 and C2, P.S. 1C and 2B, P.M. 2.1.2, Project # 5325-41440-006-00D)

A new family of bio-based lubricant additives using boron. Current lubricant additives require the use of phosphorous, zinc, and sulfur to be effective, but they can react with water to form acids that corrode engine parts and bearings, and they are often incompatible with plant (bio-based) lubricants. ARS scientists in Peoria, Illinois, in collaboration with an industrial partner, have synthesized new organic additives, made primarily from boron compounds, in formulations utilizing gel-stabilized (hardened/epoxidized), bio-based oils. Anti-wear and anti-oxidation boron additives have been shown to be highly effective with soybean oil lubricants. This development helped in the creation of new bio-based lubricants that can be used in a wide variety of industrial applications. A patent on organoboron compounds has been filed. (NP 306, C 2, P.S. 2A, P.M. 2.1.2, Project #362-41000-159-00D)

A computerized, in-orchard, apple sorting harvesting aid. Currently, both defective and sound apple fruits are not sorted at harvest, but are combined, causing significant storage losses due to the defective fruits being susceptible to pest and disease infestation. The result is costly postharvest handling to remove unmarketable fruits. ARS scientists in East Lansing, Michigan, developed an in-orchard mobile system that automatically sorts and grades apples into culls (defective), processing, and fresh-market quality fruits by measuring fruit color, size, shape, and weight using color-imaging, machine-vision technology. This system incorporates harvest aid functions to reduce safety hazards for fruit harvesters. This technology will enable apple growers to leave defective fruit in the orchard, resulting in less postharvest disease/pest problems and lower postharvest storage and packing costs, and will assure a better fruit quality inventory at the warehouse. The Michigan Apple Industry Committee is testing this novel harvesting aid in commercial orchards. (NP 306, C 1, P.S. 1C, P.M. 2.1.2, Project # 3635-43640-001-00D)

Improved winter hardy switchgrass strains have high biomass yields. Lowland switchgrass cultivars have greater biomass yield than upland switchgrass, but are largely of southern origin and are often affected by winter cold temperature injury or loss from winter kill. ARS researchers in Lincoln, Nebraska, have developed an experimental lowland switchgrass with improved winter hardiness and high biomass yield potential. The lowland strain was obtained by crossing upland and lowland plants followed by three generations of selection and breeding for winter survival in Nebraska, Wisconsin, and Illinois. In eastern Nebraska, average yields were 2.4 tons per acre greater than the best available released upland cultivar. This research demonstrates the feasibility of improving the winter hardiness of switchgrass through conventional, non-genetically modified plant breeding methods by understanding the underlying genetic diversity of native germplasm populations and utilizing appropriate traits not found in existing cultivars. The lowland experimental strain is now in the seed increase phase for potential release as a cultivar. The availability of this new strain is crucial to the success of the first commercial biorefineries being constructed in the region which need high yield biomass cultivars to reduce biofuel production costs. (NP 215, Component3, Problem Statement H; Performance Measure 6.3.1, Project No. 5440-21000-028-00D)

Genetically engineered yeast overcomes major limitation to ethanol production. A major technical barrier in the commercial conversion of biomass to ethanol is the fact that the lowest cost biomass pretreatment technology – dilute-acid hydrolysis – produces byproducts such as furfural and hydroxymethylfurfural, which inhibit yeast metabolism and ethanol production. ARS scientists in Peoria, Illinois, engineered a new inhibitor tolerant, pentose utilizing yeast strain for lignocellulose-to-ethanol conversion. A patent application has been filed. They also found that the final ethanol concentration obtained from xylose fermenting, inhibitor tolerant yeasts can be increased almost 10-fold by choosing casein as the fermentor's nitrogen source. This research overcomes a major technical barrier to commercial conversion of biomass to ethanol. (NP 213, Component 3, Problem Statement A1; Performance Measure 2.1.1, Project No. 3620-41000-147-00D)

First estimates of cellulosic biofuel potential for double cropped Midwest winter rye. Concerns have been raised over potential conflicts between land used for food and biomass production. One approach to reduce potential land use conflicts is to raise cellulosic biomass crops during the period from fall through spring, between major summer crops when land lays fallow during the winter. ARS researchers in Ames, Iowa, estimated potential biomass production from winter rye planted in the fall that could be harvested for cellulosic biofuel prior to spring planting of corn or soybean for the upper Midwest regions. Using computer simulation, the average biomass yield projected for 30 locations within the study region was 1.9 tons of dry matter per acre. Total potential annual biomass production for the entire region was projected to range from 120 to 170 million tons which would yield the energy equivalent of as much as 20 billion gallons of gasoline. These results provide biofuel producers and policymakers with estimates of double crop biofuel production that could be considered as a potential resource helping meet our domestic renewable energy needs. (NP 212, Component 2, Problem Statement C; Performance Measure 6.2.1, Project No. 3625-21610-001-00D)

Energy cost savings achieved when displacing fuel oil with switchgrass. Many renewable energy studies focus on quantifying the life cycle greenhouse gas (GHG) emissions of biofuel use without considering the economic implications. Given that biomass is a limited resource, switchgrass biomass densified by pelleting was evaluated for its potential to displace fuel oil, natural gas, and coal, as well as relative consumer energy costs. ARS scientists in University Park, Pennsylvania, with collaborators at Drexel University and Pennsylvania State University, found that switchgrass pellets were a cheaper energy source than fuel oil and could save northeast U.S. consumers \$2.3 billion to \$3.9 billion annually. Switchgrass was also found to displace more than twice as much petroleum when replacing fuel oil compared with gasoline, and is cheaper as a GHG mitigation strategy when replacing fuel oil as compared to electricity. This study highlights the importance of explicitly targeting GHG reductions and petroleum offsets so that biomass is not used for more expensive options, such as electricity generation. (NP 215, Component 3, Problem Statement J; Performance Measure 6.3.1, Project No. 1902-21000-007-00D)

Keeping ethanol producers a step ahead of antibiotic resistant microbes. Antibiotics are routinely used to control bacterial contamination in fuel ethanol plants but their use may be increasingly constrained by new strains of antibiotic resistant bacteria, or by new regulations to limit the presence of antibiotics in livestock feed (for which ethanol co-products are used). ARS scientists in Peoria, Illinois, discovered laparaxin, a polypeptide produced by *Lactobacillus paracasei*, that inhibits growth of bacteria that contaminate ethanol plants, as well as some human and animal pathogens such as *Staphylococcus aureus*. A patent application has been filed. This research provides

commercial ethanol distilleries with regulatory friendly tools for controlling bacterial contaminations. (NP 213, Component 3, Problem Statement A3; Performance Measure 2.1.1, Project No. 3620-41000-135-00D)

Capitalizing on urban biomass for bioenergy production. Utilizing biomass from urban landscapes could significantly contribute to the nation's renewable energy needs. ARS scientists in Woodward, Oklahoma, demonstrated that in years of high rainfall, it was possible to harvest from 13.5 to 19.0 metric tons of dry bermudagrass biomass per hectare planted in an urban landscape. In years with normal rainfall, 0.8 to 2.3 metric tons of dry biomass per hectare was shown to be possible. It was estimated that the City of Woodward could generate about 3,750 metric tons of dry matter in a normal rainfall year and about 6,100 metric ton in a high rainfall year if every homeowner collected their lawn thatch, lawn clippings, tree leaves, and tree prunings for bioenergy production. On the basis of a 10 metric ton yield, it is estimated that 164 million metric tons of dry biomass material collected and/or recycled in the United States from urban areas annually. Biomass from urban landscapes is an untapped resource and may represent a potential resource helping meet domestic renewable energy needs. (NP 215, Component 1, Problem Statement C; Performance Measure 6.3.1, Project No. 6216-11210-007-00)

Livestock Production (ARS Goal 2) (101, 106)

Select Examples of Recent Progress:

## **NP101**

Alternatives to conventional antimicrobials for livestock. Finding novel antimicrobials that kill multi-drug resistant pathogens is a worldwide problem for livestock industries and human medicine alike. In collaboration with Spanish scientists, ARS scientists in Beltsville, Maryland, identified a bacterial cell wall degrading protein from a virus of *Staphylococcus* bacteria that when applied externally binds and kills *Staphylococcus aureus* bacteria. The protein was then fused to lysostaphin, another protein that is lethal to *S. aureus* bacteria, and then to a third bacterial cell wall degrading protein. The combination of these three proteins effectively kills both bovine and human strains of *S. aureus*, including multi-drug resistant strains. This three protein fusion strategy, to create cell wall degrading enzymes with multiple simultaneous lethal activities, is potentially applicable to any bacteria with externally exposed cell wall components and should enable production of antimicrobials that are highly refractory to resistance development while not targeting beneficial strains of bacteria. This novel fusion protein has the potential to effectively treat persistent mastitis on dairy farms and multi-drug resistant *S. aureus* (MRSA) in hospitals and clinics. (NP101, C2, P.S. A, P.M. 1.2.1, Project #1245-31000-091-00D)

Genome copy number variation affects reproduction, tropical adaptation, and metabolism in cattle. Previously, no links between genome copy number variation (CNV) and phenotype differences in cattle were known. ARS scientists in Beltsville, Maryland, and Clay Center, Nebraska, using next generation, whole genome sequencing, completed the first comprehensive discovery of CNV in cattle in relation to phenotypic differences. CNV regions from indicine and taurine cattle were linked to genes associated with health and production traits such as fertility, parasite resistance, and feed efficiency. In related research, ARS scientists evaluated more than 700,000 genome markers in beef cattle for fertility. Evidence of CNV regions were found on all chromosomes associated with reproductive efficiency, and a DNA region was identified specific to cattle that fail to conceive. These results were validated in more than 300 *Bos indicus* × *Bos taurus* pregnant females, confirming the negative effect of the CNV related gene deletions. These findings are a major step forward in identifying components affecting genetic variation beyond typical mutations which are not accounted for in current genetic evaluation systems and will lead to greater genetic insight and greater genetic progress in cattle breeding programs. These results will also enable development of DNA marker tests to improve reproduction and production efficiencies for the beef industry. (NP101, C1, P.S. A, P.M. 1.2.3, Project #1265-31000-098-00D) (NP101, C1, P.S. A, P.M. 1.2.3, Project #5438-31000-085-00D)

Temperature humidity index as a useful predictor of live performance in heavy broiler chickens. As the temperature humidity index increases drastic declines in growth and efficiency occur in heavy broiler chickens. ARS scientists in Starkville, Mississippi, found that once building temperatures exceed a surprisingly relatively cool temperature of 20°C (68°F) for 49 day old birds, the amount of feed consumed per pound of live weight gain increases dramatically from about three pounds of feed per pound of gain (which is typical for the last two weeks of production) to in excess of six pounds of feed per pound of live weight gain at 27°C (80°F), an increase of more than 100 percent in

some production systems. This finding was significant to the poultry industry. As feed costs escalate, this research underscores the critical need to optimize cooling techniques and ventilation rates to best manage feed conversion efficiencies in heavy broiler production systems, particularly in warmer climates and warmer periods during the year. (NP101, C2, P.S. A, P.M. 1.2.1, Project #6406-32630-005-00D)

Controlling ammonia emissions and increasing nutritional efficiency in dairy cows through diet. Cows excrete urea nitrogen in urine which is rapidly converted to ammonia gas and volatized into the atmosphere. Milk urea nitrogen (MUN) testing was developed to help dairy producers and nutritionists evaluate protein levels and nitrogen use efficiency of dairy cattle diets. A large component of protein is nitrogen. ARS scientists in Madison, Wisconsin, determined that MUN is also a reliable indicator of concentrations of urea nitrogen in urine and ammonia emissions from dairy farms. Six feeding trials were analyzed to determine the relationships between feed nitrogen intake, MUN, and ammonia emissions from dairy barns. Ammonia emissions dropped between 10 percent and 34 percent when MUN levels decreased, whereas milk production and animal well-being were maintained. Feeding less dietary nitrogen would save dairy producers approximately \$740 million annually in reduced feed protein (nitrogen) costs while reducing the environmental footprint of the dairy industry through reduced ammonia (greenhouse gas) emissions. (NP101, C2, P.S. C, P.M. 1.2.1, Project #3655-31000-021-00D)

Management options for range livestock producers during drought. Dealing with drought is a major challenge for livestock producers. To provide producers with options to help address severe localized drought conditions for range beef cattle producers, ARS researchers in Miles City, Montana, evaluated the effect of early weaning calves at 80 days of age on all segments of range livestock production. Early weaning was shown to improve body weight and condition of cows through the subsequent critical winter period, particularly in young cows which are generally more adversely affected by drought and body condition. It had no effect on heifer calves that were retained for breeding replacements, and it improved growth and quality performance of feedlot steers under some management protocols. Early weaning of calves provides range cattle producers with a method to conserve drought limited forages, preserve sensitive range lands, maintain cow condition and reproductive performance, and minimize the need for significant herd reductions during periods of extreme seasonal drought. (NP101, C2, P.S. C, P.M. 1.2.1, Project #5434-31000-016-00D)

Yeast supplementation improves adaptation to heat stress and enhances the health of feedlot cattle. ARS research in Lubbock, Texas, identified yeast products as nonantibiotic alternatives that improve productivity in feedlot cattle by enhancing immune function, which in turn, lessens the need for conventional antibiotics. Further collaborative research indicated that supplementing yeast products during a period of heat stress improved feed intake and weight gain while maintaining the overall health of the cattle. Analyses also indicated that yeast supplementation appears to mitigate the negative immune response effects of dietary endotoxins in feedlot rations which are increased in grain during drought years. Mitigating endotoxins improves or maintains immune function and enhances the overall health of feedlot cattle. Collectively, these studies indicate that using yeast products as a feed supplement for feedlot cattle improves health and well-being, mitigates dietary endotoxins, and reduces the negative effects of heat stress on growth and feed intake. These benefits will improve the economic competitiveness of the feedlot industry through improved efficiencies, lower costs, and enhanced adaptation to heat stress. (NP101, C2, P.S. A, P.M. 1.2.1, Project #6208-31000-006-00D)

Improved genetic progress for poultry breeders using genomic technologies. Genomic selection of animals on the basis of their DNA provides significant benefits over traditional breeding methods that rely on familial relationships and the collection of performance data. However, genomic selection in poultry has not typically been employed due to genotyping costs, the relative economic value of individual birds, relatively high reproduction rate, and relatively short generation interval for poultry. ARS researchers in East Lansing, Michigan, in collaboration with other scientists at several academic and industry research institutions, compared commercial chickens selected by genomic selection versus traditional selection. Results demonstrate that genomic selection improves breeding accuracies by up to 100 percent depending on the trait being measured. As costs for genome sequencing and genetic testing continue to decrease, poultry breeders will be able to economically adopt genomic selection and significantly speed genetic progress for a host of economically important traits. (NP101, C1, P.S. D, P.M. 1.2.3, Project #3635-31320-008-00D)

<u>Causative mutations found affecting dairy cow fertility.</u> Extensive genotyping of U.S. dairy populations by ARS scientists has revealed portions of the genome that contain five lethal mutations causing embryonic death. ARS scientists in Beltsville, Maryland, in conjunction with the University of Illinois, discovered the causative mutations

underlying two of these recessive lethal mutations in the Holstein and Jersey cattle breeds. The HH1 mutation in Holsteins caused a deletion of a gene and the deletion induces spontaneous abortion after the first trimester. The JH1 mutation in Jerseys causes a deletion of a gene that is important in regulating proper RNA splicing, and the deletion induces spontaneous abortion during the first trimester of pregnancy. These relatively late-term abortions are particularly costly because affected cows would have been confirmed pregnant by the producer and would be receiving management appropriate for pregnant females. The subsequent abortion creates stress for the cow, increases costs, decreases efficiency, and delays the onset of the next lactation. DNA tests for both mutations are now available to producers. Results are being used to guide future mating decisions in both breeds, thus increasing reproductive efficiency in dairy cows by preventing embryonic loss. (NP101, C1, P.S. ACD, P.M. 1.2.3, Project #1265-31000-098-00D)

Reducing the risk of ovine progressive pneumonia infection in sheep. Ovine progressive pneumonia (OPP) is an incurable, slow-acting, wasting disease that affects sheep in most countries, including the United States. It is one of the most costly sheep diseases due to a 20 percent decrease in lamb weaning weights and greatly increased premature culling or death of infected breeding stock. OPP also affects Bighorn sheep populations, causing death losses and jeopardizing the sustainability of commercial sheep production in the proximity of Bighorn populations. ARS researchers in Clay Center, Nebraska, compared infection rates of sheep with differing forms of a gene known to affect susceptibility to the OPP virus. Infected ewes that were heterozygous for the OPP susceptibility gene were mated to similarly heterozygous rams. Lambs born to these ewes were then tested at 10 months of age for OPP. Lambs with one or two copies of the dominant form of the OPP gene (susceptible) had three times the infection rate of lambs without the dominant form. Producers can now use DNA testing to reduce the risk of OPP virus infection which will increase productivity and improve the health and well-being of their sheep. (NP101, C1, P.S. A, P.M. 1.2.1, Project #5438-31000-082-00D)

Improving the fertility of stored turkey semen. Storing turkey semen for up to 24 hours has long been a challenging problem for the turkey industry by increasing labor and handling costs and creating production level inefficiencies compared with other species. ARS scientists in Beltsville, Maryland, discovered that specific components of the sperm membrane (phospholipids, carbohydrates) are altered when poultry semen is stored under hypothermic conditions. Alteration of these membrane components affects the ability of stored sperm cells to fertilize eggs, resulting in the exclusive use of freshly collected (i.e., nonstored) semen for artificial insemination. A new semen extender formulation developed by ARS scientists reduces the sperm membrane alterations and improves the fertility of stored turkey semen from 30 percent to 85 percent. This is a significant advancement in semen storage technology for turkey artificial insemination practices that will improve production efficiencies, lower costs, and improve genetic performance. (NP101, C1, P.S. C, P.M. 1.2.1, Project #1265-31000-093-00D)

Chromosomal regions discovered affecting female reproductive performance and sow lifetime productivity in swine. Reproductive efficiency has a great effect on the success of pork production. Improving reproductive performance and sow lifetime productivity are high priorities for the National Pork Board. To address this challenge, ARS researchers in Clay Center, Nebraska, scanned the genome of more than 1,500 female pigs for reproductive performance traits including age at first estrus, ovulation rate, litter size, preweaning mortality, and maternal influence on birth weight. Significant chromosomal regions were identified on every chromosome and accounted for 50 percent or more of the additive genetic variation in some traits. In related work, excessive weight loss during lactation often has adverse effects on reproductive performance leading to higher culling rates, delayed estrus, and compromised production, particularly in first- and second-parity young sows. The weaning-to-estrus interval (time to rebreeding), a key industry metric used to assess sow productivity; was found to be significantly affected by DNA mutations in three genes known to be involved in fat production. Five markers were identified that predicted variation in weaning-to-estrus interval from -2.6 to +0.4 days. These findings are important because producers typically invest in excess of \$350 to develop a young gilt for breeding. Additionally, 20 to 30 percent of all commercial gilts developed fail to complete parity 1 and up to 50 percent fail to complete parity 3 in some production systems. These results will now inform DNA tests to evaluate the reproductive potential of replacement gilts prior to incurring development costs and will increase the number of females that reach parity 3 or later—the minimum threshold for sow profitability in the pork industry. (NP101, C1-2, P.S. D-B, P.M. 1.2.3, Project #5438-31000-083-00D) (NP101, C12, P.S. B, P.M. 1.2.3, Project# 5438-31000-084-00D)

<u>Exploring the bovine rumen microbiome.</u> Using next-generation DNA sequencing technologies and newly developed bioinformatic pipelines, ARS scientists in Beltsville, Maryland, systematically reviewed the diversity and population dynamics of microbes in the rumen of newborn calves, mature dairy cows, and beef steers. Collectively,

21 phyla, 31 classes, 93 families, 219 genera, and at least 1,079 operational taxonomic units in the rumen were identified. The common rumen microbiome consists of 8 phyla, 11 classes, 15 families, and 17 genera across all cattle sampled. However, the bacterial communities in the rumen of preruminant dairy calves, dairy cows, and beef steers also were clearly distinguishable. For instance, greater abundance of Fibrobacteraceae and Ruminococcaceae (fiber digesting bacteria) in the rumen of beef steers were demonstrated, which may be associated with differences in their diet and likely reflect the need for enhanced fiber-digesting capacity in beef cattle. Results of this work are important in understanding the dynamics of rumen microbial populations in cattle during rumen development and in response to management, genetic, and dietary changes. A better understanding of the rumen microbiome and its interaction with these factors is needed to optimize cattle diets and will provide opportunities to improve feed utilization and production efficiencies for the cattle industries. (NP101, C1, P.S. A, P.M. 1.2.3, Project #1265-31000-097-00D)

Humane euthanasia technique developed for swine. Swine producers are seeking improved techniques to humanely euthanize piglets—a high priority for the National Pork Board. ARS researchers in West Lafayette, Indiana, in collaboration with the National Pork Board, developed a technique involving a combination of common and readily available gases: nitrous oxide, oxygen, and carbon dioxide. This two-step technique initially anesthetizes (nitrous oxide and oxygen) and then humanely euthanizes a pig (carbon dioxide), virtually without exception when administered properly. Development of this technique for on-farm use will allow pigs to be humanely and efficiently euthanized with minimal cost and oversight. This technique offers an effective and efficient alternative to current, often objectionable, euthanasia techniques that are consistently criticized by animal welfare and associated meat industry activist groups. (NP101, C2, P.S. A, P.M. 1.2.1, Project #3602-32000-009-00D)

Light source stability increases the accuracy of commercial beef carcass quality grading cameras. Variation in ambient light sources among beef carcass-grading cameras has created grading inconsistencies in packing plants that significantly affect carcass value and create marketing inefficiencies. ARS scientists in Clay Center, Nebraska, collaborated with both instrument manufacturers and commercial beef processors to develop an accurate predictor of carcass grade for use with both carcass-grading cameras typically used in commercial beef packing plants. The predictive formula has been approved by the USDA Agricultural Marketing Service (AMS) and is being rapidly adopted by the beef packing industry. These efforts will further enhance the accuracy and implementation of instrument grading for fresh beef, thereby increasing product quality, value, and marketing opportunities for the beef industry. (NP101, C3, P.S. A, P.M. 1.2.2, Project #5438-31430-004-00D)

# **NP106**

A method to recover high value co-products from grain-based ethanol production. There is increasing production of fuel ethanol from grains in the United States; the primary co-product is corn distiller's dried grains with solubles. The quantity of this relatively low value product is increasing and is used primarily in cattle but its use for feeds is limited due to an imbalance of nutrients. ARS scientists in Aberdeen, Idaho, developed a new processing method that will produce more consistent, higher protein distiller's dried grains with solubles and two new products, a protein and a mineral source with improved nutrient profiles. Feeding trials with rainbow trout demonstrated that both products are highly digestible which is vitally important to the trout industry. This new process can be easily added to the existing process which reclaims valuable phosphorus and protein from the soluble and separates the nutrients into distinct products, thus increasing the overall flexibility, economic value, and sustainability of ethanol production. (NP 106, C5, P.S. B, P.M. 2.2.2, Project #5366-21310-004-00)

Development and validation of split pond production systems to increase catfish production. Most U.S. aquaculture production comes from large, earthen ponds. Disadvantages of traditional pond production are the need for continuous management of pond oxygen concentrations, sporadic algae related fish off flavors, losses to avian predators, difficulties in disease control, inefficient fish harvesting, and lack of tight control of water quality. ARS scientists at Mississippi State University in Stoneville, Mississippi, have addressed these constraints by modifying ponds to physically separate the fish holding function from the life support and waste treatments functions. A commercial scale system, called the split pond, has been developed and validated in Stoneville. The split pond is constructed by dividing an existing earthen pond into two unequal sections with an earthen levee and then linking the two sections with water flow. Validation studies indicate that the split pond is easy to manage and that fish production can be tripled compared with that of traditional ponds. Split ponds have now been widely adopted by the

catfish farming industry. More than 1,000 acres of commercial ponds have been built with at least an additional 1,000 acres under construction or planned. (NP 106, C5, P.S. B, P.M. 2.2.2, Project #6402-31320-001-00D)

Third generation of selectively bred yellow perch reach market size four months sooner. High performers of yellow perch strains were identified, tagged, genotyped, and selectively bred by ARS researchers in Milwaukee, Wisconsin, and their University of Wisconsin at Milwaukee partners. As a result of two generations of selection, the time needed to reach market size has been reduced from 11 to seven months. Faster growth to market size will enable producers to have multiple production cycles in a single year which increases profitability of commercial yellow perch aquaculture operations. Additionally, genetic differences in resistance to viral hemorrhagic septicemia virus have been demonstrated and will enable further genetic improvement in disease resistance. (NP 106, C1, P.S. B, P.M. 2.2.2, Project #3655-31320-002-00D)

Atlantic salmon selected for increased growth and weight released to industry. Commercial salmon producers in the eastern United States are legally required to culture stocks certified to be of North American origin. Therefore, they use stocks that are only a few generations removed from wild, unselected stocks with relatively poor performance under farming conditions. ARS researchers in Franklin, Maine, are selectively breeding salmon of North American origin for faster growth and evaluated the growth of salmon from their breeding program in commercial sea cages in collaboration with industry. A salmon line selected for increased growth, more than 50 percent larger than unselected control fish, has been developed and germplasm was released for commercial production. Genetic gain has been 7 to 10 percent per generation. Utilization of improved germplasm will increase the profitability and sustainability of coldwater marine aquaculture in the United States while providing a quality seafood product to U.S. consumers. (NP 106, C1, P.S. B, P.M. 2.2.2, Project #1915-31000-003-00D)

Development of a live attenuated vaccine and in pond vaccination platform to protect catfish against enteric septicemia. A new vaccine and vaccine delivery platform have been developed resulting in exceptional protection against Enteric Septicemia of Catfish (ESC). Based on experimental pond studies conducted under conditions similar to the commercial production setting, vaccination increased survival, fish size, and feed consumption while improving the feed conversion ratio by 40.4 percent. The improved feed conversion represented up to a 30 percent reduction in feed costs, while improved production efficiencies in vaccinated fish increased gross sales more than 100 percent. Similar results were obtained over two years of field testing. Field and laboratory research is being conducted as part of the USDA licensing process for live attenuated vaccines.

(NP 106, C2, P.S. B, P.M. 2.2.2, Project #6402-31320-002-00D)

Summer diets for hybrid striped bass. High daily summertime feeding rates can result in concentrations of ammonia in pond water that are toxic to hybrid striped bass. In response, farmers may curtail feeding or switch to a lower protein feed, both of which reduce production. With input from a Mississippi-based hybrid striped bass producer, Nature's Catch, ARS scientists in Stuttgart, Arkansas, demonstrated that a diet with higher digestible protein and fat that was supplemented with deficient amino acids maximized growth and nutrient retention and reduced ammonia waste production. Fish feed manufacturers are using new digestibility coefficients recently developed by ARS in their diet formulations for hybrid striped bass, feeding the new summer diet formulation and improving summer production. (NP 106, C3, P.S. B, P.M. 2.2.2, Project #6225-31630-006-00D)

Sex steroids linked to muscle degradation in rainbow trout. Sexual maturity affects growth and meat quality in most terrestrial livestock but little is known about their actions in fish. Understanding how sex steroids affect muscle growth and quality will enable development of strategies for more efficient fillet production. ARS researchers in Leetown, West Virginia, discovered that increased rates of muscle protein degradation occur in sexually maturing rainbow trout with high sex steroid levels, even when feeding rate is high. Subsequent studies indicated that estrogens, but not androgens, act directly in muscle to reduce protein retention by both increasing rates of protein degradation and decreasing rates of protein synthesis. These data demonstrate that harvesting before sexual maturation and sex steroid exposure should result in the most efficient production of high-quality fillets in the rainbow trout. (NP 106, C2, P.S. A, P.M. 2.2.2, Project #1930-31000-010-00D)

Lower water exchange rates save money without negative effects on health and performance of Atlantic salmon. Water availability and cost of water use are two key variables for fish production in recirculating aquaculture systems, so one research goal is to minimize the rates of water exchange while maintaining excellent conditions for fish. Atlantic salmon were reared by ARS researchers in Leetown, West Virginia, for 6 months in replicated water recirculating systems operated with either high water exchange (97.5 percent of system flow recirculated; that is, 2.5

percent new water added each day) or low water exchange (99.75 percent of system flow recirculated; 0.25 percent new water added each day). Fish performance, health, and welfare were measured. The scientists determined there were no significant differences i in growth, survival, and other fish health outcomes even though the low water flushing treatment operated with 10 times less water. Though significant differences in a variety of water quality parameters were noted, none of these parameters was outside acceptable ranges for raising salmonids. This study illustrates that Atlantic salmon perform well in recirculating systems with low water exchange and demonstrates that farmers with limited water resources can compete in salmon production. (NP 106, C2, P.S. A, P.M. 2.2.2, Project #1930-31320-001-00D)

Identification of the causative agent of a significant disease outbreak in farmed rainbow trout and development of an autogenous vaccine. Emerging pathogens are a significant threat to U.S. aquaculture. ARS researchers in Leetown, West Virginia, isolated a new gram-positive bacterial pathogen causing significant loss of rainbow trout in North Carolina. The pathogen was found to be similar to *Weissella* sp., which has been associated with recent disease outbreaks in farmed rainbow trout in both China and Brazil, and this is the first report of this pathogen in the United States. At the request of stakeholders, the scientists developed and validated an effective autogenous vaccine that is now in commercial production and in use at North Carolina farms affected by this pathogen. Early pathogen detection and the rapid development and implementation of a vaccine are aiding control efforts and reduce the likelihood of further pathogen dissemination in the United States. (NP 106, C4, P.S. B, P.M. 2.2.2, Project #1930-32000-005-00D)

Rainbow trout selected for growth on plant-based feeds have increased tolerance for soy's antinutritional factors. Fishmeal is an expensive component of many trout diets. To reduce the reliance on fishmeal-based feed, ARS scientists in Aberdeen, Idaho, have selectively bred a strain of rainbow trout that grows faster and more efficiently when fed a fishmeal-free, plant-based diet. The improved fish have adapted parts of their intestinal tract to utilize a plant-based diet more efficiently without developing the intestinal inflammation observed with conventional strains of rainbow trout fed such a diet. Increasing our understanding of how trout adapt to being fed plant-based diets will aid in the continued development of improved strains of trout. (NP 106, C1, P.S. B, P.M. 2.2.2, Project #5366-21310-004-00D)

Production and evaluation of hybrid catfish using various strains of blue catfish. Hybrid catfish production (female channel catfish crossed with a male blue catfish) has expanded greatly in the United States over the past 5 years. While channel catfish strains have been well characterized over several decades, little information is available on blue catfish germplasm regarding production traits, especially in channel × blue hybrid catfish offspring. ARS scientists in Stoneville, Mississippi, have obtained strains of blue catfish from several geographic sources and initiated a program to determine the effects of blue catfish strain and individual within strain on hybrid offspring growth and fillet yield. Significant effects of individual male and female parent on offspring performance were identified and importantly, these effects were additive. This result means that the effect of parent on offspring performance is predictable and improvements will be cumulative. Based on results of these trials, blue catfish germplasm that produces superior performing hybrid offspring will be identified and released to the industry. (NP 106, C1, P.S. B, P.M. 2.2.2, Project #6402-31000-009-00D)

<u>Pharmacokinetics of florfenicol in channel catfish.</u> ARS researchers in Stoneville, Mississippi, in collaboration with their colleagues from Mississippi State University, College of Veterinary Medicine (MSU-CVM), conducted pharmacokinetic studies of florfenicol in catfish. These studies along with laboratory and field efficacy studies were pivotal in obtaining Food and Drug Administration approval for the use of florfenicol medicated feed to treat *Flavobacterium (F.) columnare* and *Edwardsiella ictaluri* infection in catfish. In addition, these studies along with one conducted by the U.S. Fish and Wildlife Service in Bozeman, Montana, cleared the way for approval of florfenicol to treat *F. columnare* infections in all freshwater-reared warm-water fish. The drug is prescribed under a veterinary feed directive and has been shown extremely effective in controlling bacterial infections in warm-water fish. (NP 106, C4, P.S. B, P.M. 2.2.2, Project #6402-31320-002-00D)

Crop Production (ARS Goal 2) (301, 305)

**Select Examples of Recent Progress:** 

## NP301

New USDA Plant Hardiness Zone Map released. The USDA Plant Hardiness Zone Map (PHZM) is an essential tool for the country's estimated 60 million gardeners, nurseries, horticultural producers, plant breeders, and government agencies that use it as a primary reference to define geographic patterns of extreme winter cold and identify plants that are most likely to thrive in a particular area. ARS researchers from Ames, Iowa, and colleagues from the PRISM Climate Group at Oregon State University in Corvallis, Oregon, have completed a new 2012 edition of the PHZM which brought the map into the "Digital Age." It combines leading edge geographical information systems analytical technology, Internet information delivery, and traditional horticultural and climatological scientific expertise and clientele input to transform what had been a static, paper map into a dynamic, Web accessible, multi-functional interactive information resource. The new PHZM has set a new standard of accuracy and repeatability for such maps. The PHZM Web site attracted more than 500,000 visitors during the first two weeks after release and more than two million visitors to date. The PHZM earned a Special Achievement in Geographical Information Systems Award from Esri, Inc., one of 150 chosen for awards from 100,000+ Esri clients worldwide. (NP 301; C1; PS1A; PM 2.2.3; Project # 3625-21000-053-00D)

Import of genetically engineered, disease-resistant papaya to Japan approved. A major portion of the U.S. market share of papayas sold in Japan was lost when the Hawaiian papaya industry was devastated in the 1990s by the papaya ringspot virus. The first shipments to Japan of 'Rainbow,' a transgenic papaya ringspot virus-resistant variety, took place in December 2011, following final regulatory approval by the Japanese government. The 'Rainbow' import application package was prepared and submitted in a collaborative effort involving ARS researchers in Hilo, Hawaii, and researchers or representatives from several U.S. universities and the papaya industry. 'Rainbow' papaya is the first fresh genetically engineered fruit to be marketed commercially in Japan. (NP 301; C4; PS4C; PM 2.2.3; Project # 5320-21000-013-00D)

Tomato genomes sequenced. The full genome sequence for a crop constitutes a powerful tool for a wide spectrum of research goals, most importantly enabling breeders to accelerate the development of superior varieties. Tomato is one of the most important vegetables globally, as it provides key vitamins and minerals to populations worldwide, and generates billions of dollars of economic value in the United States. The International Tomato Genomics Consortium led by ARS scientists in Ithaca and Cold Spring Harbor, New York, sequenced the genomes of the domesticated tomato and its wild ancestor. These genome sequences constitute an invaluable knowledge base for uncovering the genetic bases for tomato fruit taste, quality, size, yield, nutritional content, and host-plant resistance to diseases, pests, and environmental extremes. Access to this new knowledge should enable tomato breeders and geneticists to enhance the efficiency and effectiveness of tomato crop improvement for key, high value horticultural traits. (NP 301; C2 and C3; PS2B and 2C; PM 2.2.3; Project # 1907-21000-033-00D)

More than 150 corn traits mapped to specific regions of the corn genome. ARS corn researchers in Columbia, Missouri; Ithaca, New York; and Raleigh, North Carolina, completed extensive evaluations of biochemical and agronomic traits for 5,000 corn lines that collectively included much of the total variation found in the corn breeding gene pool. These evaluations located the regions of the corn genome associated with more than 150 traits of major agronomic importance. Also, researchers characterized more than 55 million variable regions in the corn genome as a first step for identifying genetic markers for breeding those traits or the actual genes controlling the traits. All of this important new information and corn genetic stocks are accessible through the Maize Genome Database. This new information will enable variation in corn gene sequences to be associated with gene function and with variation in plant traits. The analytical (informatics) tools developed for managing those data and relating them to other genome databases for other plants will enable geneticists and breeders to more rapidly identify genes for improved crop performance, quality, and yield. (NP 301; C4; PS4B; PM 2.2.3; Project # 3622-21000-034-00D; NP 301; C1; PS1B; PM 2.2.3; Project # 1907-21000-029-00D)

Novel method for identifying different nitrogen fixing bacteria and determining their benefits to soybean growth and production. Soybean benefits from symbiotic bacteria rhizobia that occupy the roots and help the plant to produce its own nitrogen, thus eliminating the need for nitrogen fertilizer applications. The many different genetic types of rhizobia differ substantially in their ability to produce the nitrogen required for soybean growth and production. Several genetic types of rhizobia might occupy the same plant simultaneously, each with a specific preference for a different site on the soybean roots, and each with significant variation in efficiency for producing nitrogen. Consequently, identifying the unique types of rhizobia in culture collections is a priority, as is determining how

effectively the different types produce nitrogen in symbiosis with the soybean plant. ARS researchers at Beltsville, Maryland, developed novel methods for describing soybean rhizobia genotypes belonging to the genus *Bradyrhizobium*, and analyzing how these bacteria occupy soybean roots. This new technology includes a method to extract rhizobial DNA directly from sites on soybean roots to identify genetic types of rhizobia in each root location, determine nitrogen production efficiency, and identify the soybean varieties that form symbioses with the most efficient rhizobia. (NP301; C1, PS 1A; PM 2.2.3; Project # 1245-21000-226-00D

New wheat germplasm developed from wild relatives with more effective resistance genes to Ug99 stem rust. Stem rust strain Ug99 threatens wheat production worldwide. ARS researchers in Manhattan, Kansas, and St. Paul, Minnesota, along with Kansas State University collaborators, developed a wheat germplasm line with Ug99 resistance gene (Sr52) derived from a wild wheatgrass species. In addition, ARS researchers in Fargo, North Dakota, and St. Paul, Minnesota, used chromosome engineering techniques to successfully transfer small segments of goatgrass chromosomes carrying the Ug99-resistance gene (Sr47) to durum wheat. The source of the wild wheat relatives was from seeds safeguarded in the USDA Small Grains Repository in Aberdeen, Idaho. The new lines with highly effective resistance genes from the wild relatives of wheat will help wheat breeders throughout the world develop more durable protection of the global grain supply. (NP301; C3; PS3A; Project # 5430-21000-006-00D; and PS3B; Project # 5442-21000-033-00D)

Genetic control of essential dietary minerals in beans. Dry beans (*Phaseolus* species), a staple food world- wide, are a valuable source of dietary zinc and iron for populations with largely plant-based diets. ARS researchers in East Lansing, Michigan, analyzed dry bean cultivars with different concentrations of zinc and identified genes in two navy bean cultivars with divergent gene expression patterns that yielded a 30 percent difference in seed zinc concentration. They determined that 10 differentially expressed genes are involved in zinc or iron transport in dry beans. The identification of these differentially expressed transport genes will enable more effective breeding for increased seed zinc and iron levels in dry beans to benefit human nutrition. (NP301; C2; PS2B; PM 2.2.3; Project # 3635-21430-009-00D)

Genes that control the production of a key component of grape varietal aroma are mapped. Meothoxypyrazines, a class of volatile aromatic compounds, are commonly generated by grape varieties traditionally grown in Bordeaux, such as Cabernet Sauvignon and Sauvignon Blanc. At moderate to low levels, these compounds are considered a desirable and key component of wines fermented from these grapes. However, at high concentrations, these compounds are considered undesirable because they yield wines that are excessively "vegetal" in their aromatic profile. ARS researchers in Geneva, New York, demonstrated that variation in the concentration of these compounds is quantitatively inherited and controlled by two unlinked genes with large genetic effects. Mapping this trait is the first step in developing an assay that will improve the efficiency for selecting this trait during grape breeding and will lead to further insights into how developing grape berries produce these compounds. (NP 301; C2 and C3; PS2C and 3B; PM 2.2.3; Project # 1910-21220-005-00D)

<u>Citrus rootstocks that confer greater tolerance to citrus greening in sweet oranges</u>. Currently, no commercial source of resistance is known for the lethal citrus greening disease, which is widespread in Florida where it severely threatens this important industry. ARS researchers in Fort Pierce, Florida, compared tree health and fruit cropping for sweet orange trees grafted onto numerous rootstocks in four field trials conducted in a region severely affected by greening. Significant differences in rootstock tolerance of the disease indicated that some rootstocks will enable citrus trees to better tolerate greening disease as one component of successful citrus production management in the presence of that disease. (NP301; C3; PS3C; PM 2.2.3; Project # 6618-21000-013-00D)

Identifying a sorghum gene for superior livestock feed and bioenergy value. Lignin is the principal substance that makes cell walls resistant to breakdown either by livestock digestive systems or during the cellulosic bioenergy conversion process. Brown midrib 2 (*bmr2*) is a mutant gene that reduces lignin content and alters the lignin composition of sorghum cell walls. The specific genetic mutation causing *bmr2* was identified by ARS researchers in Lincoln, Nebraska, and their colleagues at the University of Florida as affecting an enzyme involved in lignin synthesis. The function of the enzyme was characterized and determined to be largely responsible for an initial step in lignin synthesis. This new information about the *bmr2* gene can be applied to manipulate lignin content in sorghum and to improve that crop as a source of animal forage and bioenergy feedstock. (NP 301; C3; PS3A; PM 2.2.3; Project # 5440-21220-027-00D)

Construction of a new high-quality cotton genetic map. ARS researchers in New Orleans, Louisiana, with their U.S. and French collaborators, have constructed a new, high-quality (termed ultra-dense) genetic map for commercial Upland cotton by combining six separate high density maps, to produce an ultra-dense map with more than 8,200 unique DNA markers. Statistical comparisons of those mapped markers with the smaller and simpler genome sequence of a wild cotton species revealed strong structural similarities between the genomes of the two species, allowing information from the simpler genome to be used to locate genes or other genetic features on the larger genome of Upland cotton. This ultra-dense map is a valuable resource for mapping the genes controlling complicated traits such as fiber yield and quality, isolating and cloning key cotton genes, and more comprehensively understanding cotton genome structure and evolution. The map will also serve as the standard reference and starting point for additional research on the structure of the genomes of different cotton populations and species. (NP301; C1, PS1A; PM 2.2.3; Project # 6435-21000-016-00D)

## **NP305**

RNAi technology -- a new strategy for controlling infectious diseases and parasites of bees. Israeli acute paralysis virus is a suspected cause of Colony Collapse Disorder (CCD). ARS scientists in Fort Pierce, Florida, in collaboration with university, industry (Beeologics/Monsanto), and military partners, have successfully developed a new control strategy that uses RNA interference (RNAi) technology to protect bees against the virus. The RNAi product protects bees when fed sugar solutions, routinely used by beekeepers as a honey substitute. This RNAi strategy not only controls the virus, and thus has potential for reducing CCD, but could also be adapted to target and control other diseases and pests of bees and other insects. This was the first large scale, field proof-of-concept use of RNAi for pest control. The bee treatment product is now commercialized as Remebee©. (NP 305; C 2; P.S. 2.A.1; P.M. 2.2.3, Project # 6618-22320-001-00D)

Improved technology for producing long cane blackberry plants. U.S. blackberry growers would like to extend the growing period of their plants to enable them to sell fruit after the traditional growing season as out of season blackberries command a higher price. ARS researchers in Kearneysville, West Virginia, used a unique trellis and cane training system to propagate long cane plants which can be manipulated to produce off season fruit. The new propagation system increased plant output five to seven fold over the current commercial propagation technique. The new innovations include long cane plants that are rooted at both ends of the cane. These long cane plants produced more fruit clusters, clusters with more fruit, larger fruit, and 250 percent increase in fruit production compared to long cane plants produced by traditional methods. The new propagation method is efficient for producing a large number of long cane blackberry plants which will be useful to both growers and nurseries. (NP 305; C 1, P.S. 1B.3; P.M. 2.2.3; Project # 1931-21000-018-00D)

Maximizing benefits for energycane and sugarcane production systems. In some areas of Louisiana, sugar production is not economical because of delayed planting or flooded conditions. ARS scientists in Houma, Louisiana, completed a five year study comparing the flood tolerance of energycane and sugarcane. Production practices vary whether sugarcane is grown primarily for sucrose (sugarcane) or as a biofuels feedstock (energycane); energycane increases management flexibility for the existing sugarcane production system. Energycane tolerated the flooded conditions better than sugarcane when biomass and sucrose yields were compared between treatments. Utilization of energycanes for production of cellulosic biomass may be a sustainable rotational crop for sugarcane. (NP 305; C 1, P.S. 1B.3; P.M. 2.2.3; Project # 6410-12210-001-00D)

Fertilizer value of recovered phosphorus from animal manures. Many fields around large animal feeding operations in the southeastern United States have excessive soil phosphorus from land applied animal manures. Previously, a method to recover phosphorus from animal waste in a concentrated form was developed by ARS scientists in Florence, South Carolina. These ARS scientists field tested the recovered phosphorus for its value as a fertilizer product, and found that the material can be processed into commercial sized fertilizer pellets with relative ease. They discovered that when it is land applied as small particles (between 0.5 and 1.0 millimeters in diameter) recovered phosphorus is effective as a fertilizer source. Since the recovered phosphorus can be transported in concentrated form and recycled as plant fertilizer, adoption of this technology by animal producers will reduce the environmental impact of excessive phosphorus in soils around these animal farms. In addition, recycling manure phosphorus will lengthen the duration of the world's supply of minable phosphorus. (NP 305; C 1, P.S. 1A.4; P.M. 2.2.3; Project # 6657-21000-006-00D)

Bees with varroa sensitive hygiene trait resistance against varroa mite commercialized. Varroa mite is the most important pest of honey bees. While miticides partially control the mite, some mites are resistant and there is need for additional tools. ARS scientists in Baton Rouge, Louisiana, found that some lines of bees remove mite-infested brood from hives. They named the trait varroa sensitive hygiene (VSH), and commercialized VSH-bee lines. Twenty-five percent of the honey bee queens now being sold contain the VSH trait. This success resulted in one of the top U.S. awards for technology transfer, a Federal Laboratory Consortium Award for Excellence in Technology Transfer in 2012 for ARS. (NP 305; C 2, P.S. 2.A.2; P.M. 2.2.3; Project # 6413-21000-013-00D)

Agroenvironment impact on mamey sapote, a tropical tree fruit. The demand for tropical fruits has increased significantly during the last decade as consumers seek healthy and more diverse food products. There is a lack of formal experimentation to determine yield performance and fruit quality traits of mamey sapote (*Pouteria sapota*) cultivars. ARS scientists in Mayaguez, Puerto, Rico, evaluated six mamey sapote cultivars ('Copan', 'Magana', 'Mayapan', 'Pace', 'Pantin', and 'Tazumal') grown on ultisol and oxisol soils for 5 years at Corozal and Isabela, Puerto Rico, respectively. The scientists found a significant difference in the number and weight of fruit per hectare between locations. 'Tazumal' had the highest 5-year mean number and weight of fruit per hectare, but fruit of this cultivar was very small and contained several seeds, which could reduce its marketability. At Corozal, cultivars 'Tazumal' and 'Magana' had significantly higher fruit yield per hectare than the rest of the cultivars, whereas 'Magana', 'Tazumal', and 'Pantin' had the highest fruit yield at Isabela. At both locations, 'Pantin' had relatively high yield, above-average soluble solids concentration values, and adequate fruit size and weight for domestic and export markets (650–900 g), making this cultivar suitable for planting at various agroenvironments typical of the humid tropics. This study provides, for the first time, valuable information to growers and Extension specialists on yield, fruit quality traits, and adaptability of mamey sapote cultivars grown in various agroenvironments. (NP 305; C 1, P.S. 1B.1; P.M. 2.2.3; Project # 6635-21000-050-00D)

New protocols for spray drift reduction. Numerous new spray technologies including nozzles, spray formulations, adjuvants, and operational practices may significantly reduce spray drift potential, a critical concern for aerial applicators. There is a need to standardize measurement and evaluation methods so as to provide science-based guidance to applicators for enhancing drift reduction. In cooperation with EPA and other research and manufacturing entities, ARS scientists in College Station, Texas, refined and tested protocols to evaluate droplet size and drift of aerially applied spray using drift reduction technologies. Objective criteria were used to quantify the performance of drift reduction technologies, and large-scale model simulations provided guidance on best operational practices such as droplet size, swath offset, and application height. These new protocols will be used by applicators to counter meteorological and other in-field conditions that can elevate drift potential. The results will be better on-target deposition of aerial sprays, less drift, and more environmentally sensitive utilization of agricultural chemicals. (NP 305; C 1, P.S. 1A.2 and 1B.2; P.M. 2.2.3; Project # 6202-22000-028-00D)

Commercializing Russian honey bee lines resistant to parasites, pests, and pathogens. Varroa mite is the most important pest of honey bees. ARS researchers in Baton Rouge, Louisiana, found honey bees in Russia with some resistance to varroa mite. Once imported to the United States, the bees were intensively selected to produce a varroa-resistant stock. The bee also was found to be very resistant to tracheal mites and American foulbrood, somewhat resistant to nosema, and to harbor fewer small hive beetles than the standard Italian bee colonies. There are two mechanisms of varroa resistance: Russian bees uncap and remove the mite from infested brood cells, and the bees control the mite through frequent auto-grooming. Tracheal mite resistance is dependent on a few major dominant genes interacting with minor genes. These advances in understanding resistance makes it possible to establish a breeding stock and, potentially, to develop marker-assisted breeding. ARS lines of Russian bees are now being used by the bee industry, with 14 commercial bee breeders having certified pure Russian stock. Several commercial honey producer/pollinators also have Russian queens and are creating their own breeding stock. Russian queens play an integral role in a pesticide-resistance management program by controlling multiple hive pests and reducing the pressure to use miticides. (NP 305; C 2, PS 2.A.2; PM 2.2.3, Project # 6413-21000-012-00D)

<u>Ultra-violet light as a non-chemical plant growth regulator</u>. Traditional floriculture crop production relies upon synthetic plant hormones or hormone derivatives for controlling plant growth and development. ARS scientists in Wooster, Ohio, found that a single dose of UV light on seedlings of 10 crop species decreased photosynthesis immediately after exposure and it remained low for 1 to 10 days. Leaf expansion also decreased leading to reduced overall growth in the following weeks. Depending on the species, UV light suppressed growth in a predictable manner, similar to a chemical plant growth regulator (lower photosynthesis, decreased leaf expansion, changed

height, and no delay in flower development). This research provides a new method for non-chemical plant growth regulation. (NP 305; C 1, P.S. 1C.4; P.M. 2.2.3; Project # 3607-21000-015-00)

Bee health possibly affected by pesticide-fungicide and pesticide-pathogen interactions. In a comprehensive survey, ARS scientists and university collaborators found 98 pesticides in the beeswax and pollen of dying and healthy colonies alike, revealing the complexity of pesticide interactions. The study highlighted the persistence of some products used to control hive pests, especially miticides for control of varroa mite. The sublethal effects of pesticides and their synergistic interactions with other bee mortality factors are prime suspects in Colony Collapse Disorder (CCD). Two results of ARS research support the need to continue this line of inquiry:

- A fungicide (Vanguard) used by almond growers in California was found to interact with a miticide (Hivastan, fenpyroximate), to increase bee mortality. These results, from ARS scientists in Weslaco, Texas, support previous findings that pesticide-fungicide interactions can overload cytochrome P450 detoxification mechanisms in bees, and suggests that one or the other product (i.e., either the fungicide or miticide) should not be used during bloom.
- Honey bee adults exposed to sublethal doses of imidacloprid (a neonicotinoid insecticide) developed higher levels of nosema spores than did nonexposed bees. The research, performed in Beltsville, Maryland, showed reduced queen emergence and higher virus levels in colonies fed virus-contaminated pollen. Thus, pesticides, even at sublethal exposure levels, might affect honey bee colony collapse, adding important evidence to the growing consensus that maladies such as CCD might be the result of a synergism of multiple factors. (NP 305; C 2, PS 2.A.2; PM 2.2.3, Project # 1245-21000-090-00D)

Food Safety (ARS Goal 4)

#### **NP 108**

# **Select Examples of Recent Progress:**

<u>Listeria monocytogenes</u> in the ready-to-eat foods. Significant efforts have been made to control *Listeria monocytogenes* in foods over the past decade. Outbreaks of foodborne illness are especially associated with ready-to-eat foods such as deli meats, soft cheeses, raw and smoked fish, and raw or partially processed vegetables. At the request of the FDA and the FSIS, ARS researchers in Wyndmoor, Pennsylvania, undertook a study to determine the current prevalence and levels of *Listeria monocytogenes* in deli packaged versus pre-packaged ready-to-eat foods purchased at retail establishments in four FoodNet sites. The study indicated an observed *Listeria monocytogenes* prevalence from 0 to 1 percent for seven product categories. This is the most comprehensive survey of *Listeria monocytogenes* in retail ready-to-eat foods in the past decade, providing data critical for control for this pathogen. The study received the FDA Commissioners Award. (NP108, C 1, P.S. 1.A, B, C, D, P.M. 4.1.1, Project #1935-41420-015-00D)

Latex agglutination tests for six pathogenic non-O157 Escherichia coli. Certain Shiga toxin-producing Escherichia coli (STEC) serogroups, including E. coli O26, O45, O103, O111, O121, and O145 cause a similar illness in humans as E. coli O157:H7. The USDA Food Safety and Inspection Service (FSIS) recently declared these STECs as adulterants in beef. At the request of the FSIS, ARS researchers in Wyndmoor, Pennsylvania, developed and validated a rapid and simple testing method for confirming presumptive positive non-O157 STECs to better protect the food supply and consumers. The reagents and test protocols were transferred to FSIS for validation, and the latex agglutination method has now been incorporated into the FSIS Microbiology Laboratory Guidebook Chapter 5B.02. Adoption of this method contributed to the implementation of the USDA zero tolerance policy for the six non-O157 STECs in June 2012. The scientists who developed the LAT technology received the USDA Secretary's Award. (NP108, C 1, P.S. 1.C, P.M. 4.1.1, Project #1935-42000-056-00D)

Novel probiotics target human food safety pathogens and improve poultry health. Campylobacter and salmonella are the most commonly reported bacterial pathogens causing foodborne infections in the United States. Epidemiological evidence has implicated poultry products as a significant source of these pathogens. A novel probiotic method was developed by ARS in Fayetteville, Arkansas, capable of inhibiting growth of specific enteric pathogens. These probiotic cultures (composed of non-pathogenic healthy bacteria) target salmonella and campylobacter in the gastrointestinal system of poultry. This discovery was licensed to an Arkansas-based start-up

company in cooperation with the University Arkansas. The commercial product (FloraMax-B11) is marketed in 16 countries with approximately 300 million birds dosed per/year. (NP 108, C.1., P.S. 1.D., Project 6226-32000-011-00D)

Inactivation of Escherichia coli O157:H7 (ECOH) and non-O157:H7 Shiga toxin-producing E. coli (STEC). Escherichia coli O157:H7 (ECOH) and non-O157:H7 Shiga toxin producing E. coli (STEC) are the cause of many outbreaks of illnesses and deaths. Infections are generally foodborne with ground beef a major conduit. ARS researchers in Wyndmoor, Pennsylvania, evaluated the fate of E. coli O157:H7 and non-O157 strains in both flattened and wafers of ground beef in a heated water bath and commercial grills. Studies showed that regardless of the level of fat or type of heat/grill used, cooking ground beef patties to an internal temperature of ~71.1 degrees C was effective for destroying the pathogens. These data were transferred to the USDA Food Safety and Inspection Service for their use in consumer-related advice for safe food handling. (NP108, C 1, P.S. 1.A, B, C, D, P.M. 4.1.1, Project #1935-41420-015-00D)

Pathogens in produce growing areas in Salinas Valley, California. Several bacteria have been linked to produce associated foodborne illness outbreaks. ARS researchers in Albany, California, in collaboration with the FDA and National Aeronautics and Space Administration, conducted a survey of the Salinas watershed for the presence of *E. coli* O157, non-O157:H7 Shigatoxin positive *E. coli* (STEC), salmonella, listeria, and campylobacter. Data collected indicated substantial differences in the prevalence of the various pathogens with a definite correlation to sampling region and date. The data enabled the development of a predictive geospatial risk assessment model (PGRAM). The study provided industry and public health regulatory agencies with valuable epidemiological data for development of a risk assessment model for this important agricultural region. (NP108, C 1, P.S. 1.A, B, E, P.M. 4.1.1, Project #5325-42000-046-00D)

Rapid, portable test for botulinum neurotoxins. Produced by the common soil bacterium *Clostridium botulinum*, botulinum neurotoxins (BoNTs) are potent toxins that can cause severe foodborne disease, botulism, and could be used as a biological threat agent. ARS scientists in Albany, California, developed a rapid, sensitive diagnostic test for BoNTs that could be used by minimally trained personnel in the event of a foodborne outbreak or a bioterrorist threat. The simple lateral flow device, similar in design, use, and time as a pregnancy test, can detect and distinguish between BoNT/A and B, two of the four serotypes that are known to poison humans and together account for more than 80 percent of naturally occurring botulism. This rapid diagnostic method, which has been validated and now transferred to regulatory and other biosecurity/military agencies, is a valuable tool in the areas of food safety and homeland security. (No national program info sent with this accomplishment.)

<u>Vaccine development for E. coli O157:H7</u>. E. coli O157:H7 can cause life threatening foodborne illnesses. Beef cattle are a major asymptomatic carrier of the pathogen, and development of a vaccine for cattle to eliminate the pathogen is a major goal for government and industry. E. coli O157:H7 colonize the terminal portion of the large intestine in cattle by sticking to a specific type of tissue. Specific bacterial proteins are required for adherence; studies have implicated the protein (intimin) responsible for adherence. However, ARS researchers in Ames, Iowa, have now determined that E. coli O157:H7 lacking the intimin protein use additional proteins for adherence. This finding is significant in the context of developing efficacious vaccines for blocking adherence of the bacteria. Better vaccines would be those that would include not only the intimin protein but other proteins to reduce adherence. ARS will redirect its vaccine development studies to address this critically important observation. (NP108, C 1, P.S. 1.E, P.M. 4.1.1, Project #3625-32000-100-00D)

Correctly detecting Salmonella in foodborne outbreaks. Salmonella species remain one of the leading pathogens causing outbreaks of illness. Unfortunately, the serotype implicated in actually causing any outbreak (clinical disease) is often difficult to determine since there may be many contaminating strains. During outbreak investigations it is critical to isolate the relevant strain from food and/or environmental sources. ARS researchers in Albany, California, determined that some Salmonella strains were more likely to be isolated than others. Current selective enrichment media shows a bias for Salmonella enterica strains while strains of serogroup B, which include serovars Typhimurium, Saint-Paul, and Schwarzengrund, were less likely to emerge as dominant strains. This work provides critical information to public health agencies at the Federal and State level, as well as to industry, stressing that during investigations, multiple enrichment protocols should be used to ensure isolation of target strains. (NP108, C 1, P.S. 1.A, C, P.M. 4.1.1, Project #5325-42000-046-00D)

Genomic markers for identifying specific pathogenic Escherichia coli strains. The USDA Food Safety and Inspection Service recently declared Escherichia coli strains O26, 045, O103, O111, O121, and O145 adulterants in beef trim and started regulatory screening for these pathogens. The current method for detecting these specific (serotypes) takes several days because there is not a specific genomic marker for each serotype. ARS scientists in Clay Center, Nebraska, identified strain specific DNA markers for each serotype by comparing portions of the DNA. The DNA markers were licensed to a company that makes diagnostic kits for foodborne pathogens and are being used as part of a commercially available assay. This assay will be useful for industry and government researchers. (NP 108, C.1., P.S. 1.C., Project 5438-42000-015-00D)

<u>Cronobacter sakazakii</u> in powdered infant formula. Cronobacter sakazakii is a deadly foodborne pathogen found in dehydrated powdered infant formula. ARS researchers in Wyndmoor, Pennsylvania, investigated the growth kinetics of *C. sakazakii* in reconstituted powdered infant formula and developed predictive models. Thermal growth studies indicated that *C. sakazakii* grows well at temperatures between 10 to 48 degrees C. The results will assist industry in their production of infant formula, regulatory agencies in conducting risk assessments of reconstituted powdered infant formula exposed to various temperature-abuse conditions, and parents and other caretakers in properly storing and preparing reconstituted powdered infant formula. (NP108, C 1, P.S. 1.E, P.M. 4.1.1, Project #1935-42000-075-00D)

Natural antimicrobials to replace antibiotics in swine diets. The use of antibiotics in animal production is a controversial issue due to the concern of transmission of antibiotic resistance genes. Young swine are often fed dietary antibiotics to improve health, reduce pathogen load, and enhance performance. Few natural alternatives have been identified to replace these compounds. ARS scientists in Clay Center, Nebraska, determined that a commercial product containing lysozyme (naturally found in eggs) could replace dietary antibiotics. The impact of this research, particularly for industry, is that the use of lysozyme in diets of young piglets could help maintain a safe food supply and reduce the use of prophylactic antibiotics that are typically used for swine production. (NP108, C 1, P.S. 1.A, D, P.M. 4.1.1, Project #54385-32000-030-00D)

Livestock Protection (ARS Goal 4) (103, 104)

Select Examples of Recent Progress:

# **NP103**

Genetic evolution of novel reassortant swine influenza viruses with the capability of infecting humans. Swine influenza A virus causes a respiratory disease in pigs and variant strains that attain the ability to infect humans pose a significant public health threat. In collaboration with the National Institutes of Health, ARS scientists in Ames, Iowa, investigated the genetic evolution of novel reassortant swine influenza A viruses detected in the United States and Canada between 2009-2011 with a focus on H3N2 viruses. Analyses included H3N2 viruses designated A(H3N2) variant (v) because of their capability to also infect humans as discovered in the United States in July 2011. Analyses of samples from 12 human cases revealed that the variant swine lineage H3N2 viruses contained the pandemic matrix (pM) gene from pandemic H1N1 viruses. The A(H3N2)v viruses are distinct from contemporary H3N2 circulating in humans and the flu viruses incorporated in the human seasonal flu vaccine, and hence represent a potential pandemic threat. Monitoring and reporting evolutionary dynamics of gene segments in swine at a detailed level is critical to understanding how these novel H3N2 viruses emerged in swine and to assessing and predicting the potential epidemic and/or pandemic threat of variant influenza viruses pose to humans. (NP 103, C1, P.S.1B, and P.M. 4.2.1, Project #3625-32000-108-00D)

<u>Seeking alternative strategies to antibiotics</u>. Although widespread use of antibiotic-based growth promoters has improved the efficiency of worldwide poultry production, there is an increasing interest in developing alternative strategies to antibiotics to control infectious diseases in livestock and poultry because of the emergence of drug resistant pathogens. ARS scientists in Beltsville, Maryland, investigated dietary phytogenics (cinnamon, garlic, and aloe vera) to enhance poultry immunity using avian coccidiosis as a disease model. Phytogenics are a group of natural growth promoters derived from herbs, spices, or other plants, and many medicinal foods and herbal products highly effective in enhancing host defense against microbial infections. ARS scientists previously showed that phytogenics augment host immunity against infectious agents and decrease inflammatory diseases. For example,

cinnamon has been shown to possess antioxidant properties and antimicrobial activities, as well as being able to modulate the immune response. In chickens fed a diet supplemented with cinnamon, the levels of certain immune cytokines were 2- to 47-fold higher compared with chickens given a non-supplemented diet. Importantly, dietary cinnamon was shown to attenuate bodyweight loss caused by *Eimeria*, an important intestinal parasite of poultry. (NP 103, C2. P.S. 2D, and P.M. 4.2.2, Project #1245-32000-097-00D)

<u>Diagnostics to detect a newly emerging virus</u>. HoBi-like virus is a newly emerging type of virus distantly related to bovine viral diarrhea virus (BVDV) that has been isolated from cattle in South America, Southeast Asia, and Europe. The clinical presentation following infection with this type of virus is very similar to that seen following infection with BVDV. Like BVDV, HoBi-like viruses cause immune suppression and can establish lifelong persistent infection in cattle. HoBi-like viruses have not yet been detected in the United States. ARS scientists in Ames, Iowa, have developed tests to provide diagnosticians and regulatory agencies with tools to screen imported animals and animal products to prevent introduction of HoBi-like viruses into the United States. These tests have been transferred to APHIS and provide the means to detect and control an introduction if it were to occur in the United States. (NP 103, C1, P.S.1B, and P.M. 4.2.2, Project #3625-32000-106-00D)

A genetic marker associated with reducing susceptibility to porcine reproductive and respiratory syndrome. Porcine reproductive and respiratory syndrome (PRRS) virus is the most important infectious endemic disease of pigs. A genetic marker for reduced susceptibility to PRRS, the most economically significant disease in pigs, has been discovered by a research team that includes ARS scientists in Beltsville, Maryland, and Kansas State University and Iowa State University scientists. This project was funded by the National Institute for Food and Agriculture (NIFA). PRRS affects pigs at all stages of growth and is easily spread. PRRS costs the United States an estimated \$642 million per year. The PRRS Host Genetics Consortium (PHGC) was established with funds from the National Pork Board to discover the genetic basis of host resistance or susceptibility to PRRS virus infection. Groups of 200 commercial crossbred pigs were infected with PRRS virus and followed for 42 days; blood samples and body weights were collected for detailed viral load and weight gain phenotypes. The entire genome of all pigs from the first three PHGC trials were searched to identify chromosomal segments that were common to pigs that had lower virus levels and faster growth after infection. This resulted in the discovery of the genetic marker, called a quantitative trait locus (QTL), on swine chromosome 4 (SSC4) associated with improved growth of pigs that are infected with the PRRS virus. These results could have a major impact on the swine industry by enabling geneticists to develop plans for marker assisted selection of pigs with improved response to PRRS. (NP103, C4, P.S. 4B, and P.M. 4.2.2, Project #1245-32000-098-00D)

Gold and nanotechnology bring viruses to light. Gold nanoparticles have the ability to scatter and absorb light, making them ideal in detecting virus infected cells. Using a technology called surface enhanced raman scattering (SERS), signals emitted from these nanoparticles can be measured using a spectrometer. ARS scientists in Manhattan, Kansas, and collaborators at the University of Wyoming used gold nanoparticles to design tests to rapidly identify West Nile virus, a virus spread by infected mosquitoes that can cause headaches, fever, flu like symptoms, and sometimes fatal neuroinvasive disease—aseptic meningitis, encephalitis, or acute flaccid paralysis. The goal of the project is to bring laboratory level analytical sensitivity to the field for portable care devices. If successful, veterinarians and medical doctors will be able to take a blood sample, put it in a small vile and read it with a hand held device. ARS scientists are also working on adapting this technology to identify multiple disease agents. (NP 103, C1, P.S.1A, and P.M. 4.2.2, Project #5430-32000-002-00D)

Development of a monoclonal antibody specific for a Johne's disease protein. Johne's disease (Paratuberculosis) is a chronic, progressive enteric disease of ruminants caused by infection with *Mycobacterium avium paratuberculosis* (MAP). Cattle become infected as calves, yet usually do not develop signs of diarrhea and weight loss until two to five years of age. During the subclinical phase of disease, animals may intermittently shed the organism in their feces, thereby contaminating the environment and infecting other animals within the herd. Current diagnostic tests are not able to accurately identify these subclinically infected animals. In order to prevent the further spread of this disease improved diagnostic tools for the detection of infection, and development of new vaccines to enhance control strategies is needed. ARS researchers at the National Animal Disease Center, Ames, Iowa, identified a new monoclonal antibody that selectively detects MAP and not other closely related bacterial strains. This antibody is currently the only one in the world that has this capability. Further research identified the protein, termed MAP1025 that this specific antibody. This antibody is the subject of a recently issued U.S. patent. The identification of this unique protein and antibody for MAP will be helpful in developing new diagnostic tools to detect infected animals. (NP 103, C5, P.S. 5A and P.M. 4.2.2, Project #3625-32000-110-00D)

Discovery of a new interferon and potential application in control of food and mouth disease. Foot-and-mouth disease (FMD) is one of the most serious threats to the livestock industry. Despite the availability of vaccines, recent outbreaks in disease-free countries have demonstrated that development of novel FMD control strategies is imperative. ARS scientists at the Plum Island Animal Disease Center reported the identification and characterization of bovine (bo) interferon lambda 3 (IFN-λ3), a member of the type III IFN family. Expression of boIFN-λ3 using a replication-defective human adenovirus type 5 vector (Ad5-boIFN-λ3) yielded a glycosylated secreted protein with antiviral activity against FMD virus (FMDV) and vesicular stomatitis virus in bovine cell culture. Inoculation of cattle with Ad5-boIFN-λ3 induced systemic antiviral activity and up-regulation of IFN stimulated gene expression in multiple tissues susceptible to FMDV infection. The result of these studies also demonstrated that the type III IFN family is conserved in bovines and boIFN-λ3 has potential for further development as a biotherapeutic candidate to inhibit FMDV or other viruses in cattle. (NP 103, C1, P.S. 1A, and P.M.4.2.2, Project #1940-32000-057-00D)

Proposal for a unified nomenclature and classification system of Newcastle disease virus genotypes. Virulent Newcastle disease viruses (NDV) are found in most countries of the world. Although the United States has strict rules to prevent their entry it is important to monitor and characterize viruses that are a potential threat to the U.S. poultry industry. ARS scientists in Athens, Georgia, have obtained strains of NDV from Mexico, Indonesia, Malaysia, Venezuela, Pakistan, Vietnam, Belize, Dominican Republic, South Africa, Peru and from wild birds in the United States and have sequenced and characterized them genetically. The sequences of key genes have allowed the prediction of the virulence of those viruses. This characterization has led to the identification of viruses of Asian lineages for the first time in the American continent (in Peru and in Venezuela) and to the identification of the expansion of the host range of North American virulent Newcastle disease from cormorants to other wild birds and the discovery of this type of viruses on the East Cost of the United States (Massachusetts, Maine, New Hampshire, and Maryland). The discovery of novel NDV on the American continent provides an opportunity to improve the classification of these viruses. Historically, two systems have been simultaneously used to classify NDV isolates into lineages or genotypes, generating confusion in the nomenclature and discrepancies in the assignment of genetic groups. Based on the extensive characterization of NDV collected worldwide, ARS scientists have proposed a unified nomenclature and a classification system based on objective criteria to separate NDV into genotypes resulting in distinct taxonomic groups. Results revealed that class I viruses comprise a single genotype, while class II contains 15 genetic groups including 10 previously established (I-IX, and XI) and 5 new genotypes (X, XII, XIII, XIV and XV). Adoption of a unified nomenclature and of objective criteria to classify NDV isolates will facilitate studies on NDV epidemiology, evolution, disease control and diagnostics. (NP 103, C1, P.S. 1B, and P.M.4.2.1, Project #6612-32000-064-00D)

The U.S. Veterinary Immune Reagent Network. The U.S. Veterinary Immune Reagent Network (VIRN) (www.vetimm.org) was established to address the lack of immunological reagents specific for livestock and poultry species. Efforts are targeted at swine, ruminants, poultry, equine and aquaculture species. ARS scientists in Beltsville, Maryland, have led the teams for swine and poultry and have successfully developed and characterized bioactive immune proteins, cloned cytokine and chemokine proteins, as well as monoclonal antibodies (mAbs) to these proteins and their receptors and immune cells. These reagents will be used to evaluate swine and poultry immune responses, changes after infections or following vaccination, and give scientists the ability to manipulate these immune proteins, and cell populations to evaluate their roles in protective immunity, immunoregulation, and immunopathology. Recombinant cytokines and chemokines for swine and poultry were cloned and expressed in yeast, purified and shown to be bioactive. All immune proteins developed in this proposal are available to collaborators and have been made commercially available through the U.S. VIRN partner, Kingfisher Biotech, Inc. <a href="https://www.kingfisherbiotech.com/">www.kingfisherbiotech.com/</a>. Another goal is to produce mAb reagents that function in different diagnostic platforms. Overall the U.S. VIRN projects are important as a means of identifying new reagents and technologies for veterinary diseases and diagnostic and vaccine discovery research. (NP 103, C2, and P.M. 4.2.2, Project #1245-32000-097-00D, 1245-32000-098-00D)

<u>Determination of an effective bison Brucellosis vaccine strategy.</u> Brucellosis is a disease of production livestock that can cause devastating economic losses to producers and can also cause severe illness in humans. Since being initiated in 1934, billions of dollars have been spent on the U.S. Cooperative Brucellosis Eradication Program in efforts to eradicate the disease. However, the persistence of brucellosis in wildlife reservoirs (bison, elk, feral swine, and other species) and in neighboring countries poses a risk for reintroduction to domestic livestock. Although all 50 states have maintained brucellosis-free status for cattle since 2009, the infection of 3 herds in Wyoming in 2010 demonstrates the risk associated with wildlife reservoirs of *B. abortus* in the Greater Yellowstone Area. There is a

high prevalence of brucellosis in free-ranging bison in Yellowstone National Park. Any vaccination program of bison will be difficult and expensive so determination of the most efficacious brucellosis vaccine strategy for bison is needed. ARS researchers in Ames, Iowa, evaluated the safety, immunity, and protection after bison were vaccinated with a single RB51 injection, vaccinated four times by injection, or a single pneumatic vaccination with a needleless system. Measurements of immunity were similar across treatments and did not support the hypothesis that multiple vaccinations would induce greater responses. Experimental protection was similar for the single injection and pneumatic vaccination treatments, but data suggested that bison receiving multiple vaccinations had reduced protection. These data suggest that too frequent vaccination of bison calves with RB51 may actually reduce protection against brucellosis. In addition, results from this study suggest that pneumatic vaccination can be a safe, effective, and needle-less procedure for disease prevention in bison. (NP 103, C3, P.S. 3A, and P.M. 4.2.2, Project #3625-32000-111-00D)

Development of blood based diagnostic tests for bovine tuberculosis. Sine the USDA initiated a bovine tuberculosis eradication campaign in 1917, significant progress has been achieved, but eradication has proved elusive. Obstacles to eradication include lack of rapid, sensitive and specific blood based diagnostic tests for cattle and the wildlife reservoirs which serve as a source of tuberculosis infection for cattle. Development of improved tuberculosis (TB) diagnostic tests serves the both the cattle and captive cervid industries. Novel diagnostic tests allowing testing for TB in a single handling event, thus decreasing stress and potential for injury to animals or humans as well as speed of results is critical in meeting this important need. ARS scientists in Ames, Iowa, developed two new blood based assays including a Enzyme-Linked Immunosorbent Assay (ELISA) for diagnosing TB in cattle and white tailed deer. This will increase the ability to quickly diagnose infected animals that will assist in eradication of this important zoonotic disease. (NP103, C3, P.S. 3C, and P.M. 4.2.2, Project #3625-32000-104-00D)

Development of a Pasteurella multocida vaccine for cattle respiratory disease. Respiratory disease of beef and dairy cattle is considered the most costly disease facing producers today with estimated costs exceeding \$1 billion annually. A recent National Animal Health Monitoring System (NAHMS) survey confirmed that respiratory disease continues as the leading cause of morbidity and mortality in U.S. feedlots and is the most common cause of weaned dairy heifer mortality. The availability of new, more effective measures to prevent and control respiratory disease threats will have a significant impact on the future of the cattle industry. The etiology of Bovine Respiratory Disease (BRD) is complicated by numerous factors of stress and multiple bacterial and viral infections. Normal cattle may carry one or more of the bacterial and viral agents in their upper respiratory passages with no apparent ill effects. Some of these agents produce only mild clinical signs by themselves, but when combined with other viral or bacterial agents and/or stress they may cause severe clinical disease and even death. Pneumonic pasteurellosis caused by Pasteurella multocida is an ongoing cause of multi-million dollar losses to the beef and dairy cattle industries. Two new modified-live vaccine strains of P.multocida were constructed by ARS researchers in Ames Iowa, which may be delivered to calves by conventional injection or by a intranasal route. Effective vaccines to control P. multocida in susceptible cattle populations will save livestock producers millions of dollars annually while reducing therapeutic antibiotic usage and increasing the quality of beef products. (NP103 C4, 4A, and P.M. 4.2.2, Project #3625-32000-105-00D)

Reduced bacterial clearance from the lungs of bighorn sheep. Respiratory pathogens of domestic sheep including *Mannheimia haemolytica (Mh)* lead to millions of dollars in production losses annually. In addition to production losses in domestic sheep, *Mh* is an important bacterial pathogen of bighorn sheep. Data indicate that domestic sheep transmit pathogenic subtypes of *Mh* to bighorn sheep, and a currently debated intervention is separation of domestic from bighorn sheep areas. However, the current separation strategy consisting of removing domestic sheep grazing rights in the West economically threatens a large fraction of the U.S sheep industry, and additional intervention strategies are urgently needed. Researchers from Washington State University along with ARS scientists in Pullman, Washington, administered equivalent dose of *Mh* simultaneously to bighorn and domestic sheep and the bighorn sheep failed to remove the bacteria from their lungs as quickly or as thoroughly as the domestic sheep. As a result, bighorn sheep tend to have more severe lung damage associated with *Mh* pneumonia than domestic sheep, but the reasons for lack of bacterial clearance is not clear. These findings did not rule out an overactive immune response as part of the explanation for differing lung damage between these closely related species. However, the differences in bacterial clearance between the two sheep species provide baseline data for future studies to determine potential mechanisms for the severity of respiratory disease in bighorn sheep. (NP103, C4, P.S. 4A and P.M. 4.2.1, Project #5348-32000-031-00D)

Genetic resistance to nematode infection in cattle. Gastrointestinal nematode infections in ruminants remain a major impediment to the efficient production of both meat and dairy products and an important factor in constraining global food availability. Effective parasite control strategies are currently limited and based largely upon heavy drug usage. The treatments can be expensive and labor intensive, thus reducing profitability. There has been a recent increase in parasite resistance to anti-parasitic drugs making development of new strategies imperative. In order to develop new and novel strategies, the use of bioinformatic tools enables us to better understand host-parasite relationships. ARS scientists in Beltville, Maryland, characterized the response of the abomasal transcriptome to gastrointestinal parasites in parasite-susceptible and parasite-resistant Angus cattle using RNA-seq technology. These cattle displayed distinctly separate resistance phenotypes as assessed by fecal parasite egg counts. After assessing 15,432 bovine genes expressed in the abomasum, 64 genes were identified as significantly over-expressed in resistant cattle. Several specific biological pathways were found to be impacted in resistant animals suggesting their potential involvment in the development of parasite resistance. Understanding the mechanisms used by either the host or the parasite to resist or reduce parasites will enable the development of alternative strategies of control, one of which could include using genetic selection in the host. (NP 103, C6, P.S. 6A and P.M. 4.2.1, Project #1245-32000-093-00D).

Determination of a new tick vector for equine babesiosis. The insidious reemergence of equine babesiosis or piroplasmosis in 2009 in Texas is a poignant reminder of the vigilance required to maintain an infection-free status in the United States. Equine piroplasmosis has been considered a foreign animal disease in the United States since 1980's. Ticks native to the United States such as *Dermacentor variabilis* and *Riphicephalus microplus* are known vectors for *Babesia equi*, the causative agent of equine babesiosis. ARS scientists in Pullman, Washington, in collaboration with APHIS-National Veterinary Service Laboratory in Ames, Iowa, and APHIS-Western Regional Office, Fort Collins, Colorado, identified the native U.S tick, *Amblyomma cajennense*, as the predominant tick species found on horses at the Texas outbreak and capable of transmitting *B. equi*. In addition, it has the potential to geographically expand within the United States due to acaricide resistance and distribution by infected horses and wildlife adding an additional risk factor. Persistently infected horses are the reservoirs for tick acquisition of *B. equi* and its transmission to susceptible horses. Understanding the competency of the U.S. native ticks is critical to designing an efficient control strategy to prevent the dissemination of equine piroplasmosis within the United States and avoid great economic burdens to the U.S. horse industry. (NP103, C6, P.S. 6B, and P.M. 4.2.1, Project #5348-32000-034-00D)

Pathologic and biochemical characterization of a genetic form of bovine spongiform encephalopathy. Bovine spongiform encephalopathy (BSE) is a transmissible spongiform encephalopathy (TSE) of cattle, and was first diagnosed in 1986 in Great Britain. There have been four cases diagnosed in the United States, the first was in December 2003 in a cow imported from Canada, and the three subsequent cases were found in cows born and raised in the United States. The 2003 imported case is reported to have cost the U.S beef industry \$3.2 to 4.7 billion. The first U.S. indigenous case was found in a downer cow in Texas in November 2004; the second was found on a farm in Alabama in March 2006; and the third was case was found in 2012 in a dairy cow in California. One of the cases was shown to be genetic and inheritable. The genetic form of BSE is analogous to the most prevalent hereditary form of human TSE. Heritable BSE along with spontaneous BSE forms are also referred to as atypical BSE cases which have important implications in that they are not associated with the feedborne epidemic of classical BSE first recognized in the United Kingdom in the 1980's. The complete pathologic and biochemical features of the U.S genetic form of BSE was defined and reported for the first time by ARS scientists in Ames, Iowa. The pathologic and biochemical characterization of this genetic atypical BSE case will help greatly with detection and identification should future cases arise in the U.S. (NP 103, C7, P.S. 7A, and P.M. 4.2.1, Project #3625-32000-103-00D)

A genetic marker associated with resistance to scrapie. Scrapie is a fatal neurodegenerative disease of sheep and goats. Its economic impact on the sheep industry is such that the USDA administers a voluntary eradication program in collaboration with the sheep industry. The amino acid, lysine, at position 171 of the sheep prion protein delays development of scrapie. Demonstrated the affect of the amino acid lysine at position 171 of the sheep prion protein on susceptibility to scrapie, a transmissible spongiform encephalopathy of sheep. Amino acid differences in the prion protein are known to play a major role in scrapie susceptibility in sheep and these genetic differences are utilized in the strategy to remove scrapie from our nation's sheep flock. Natural scrapie had previously only been described in one sheep with lysine at position 171 of the prion protein, hence not enough information was available from natural cases to determine the affect of lysine at position 171 on scrapie susceptibility. ARS scientists in Ames, Iowa, demonstrated that sheep with a prion protein containing lysine at position 171 are susceptible to scrapie but have a prolonged scrapie incubation period, and that the abnormal prion protein accumulates throughout the central

nervous system and lymphoid organs. Because sheep with lysine at prion amino acid position 171 develop scrapie at a slower rate than other known susceptible genotypes this information is critical to sheep breeders that want to eradicate genotypes susceptible to scrapie. (NP 103, C7, P.S. 7D, and P.M. 4.2.2, Project #3625-32000-103-00D)

Development of a rapid method for detection of disease-associated prions. The rapid detection of transmissible spongiform encephalopathies (TSEs) is a priority for the U.S. livestock industries. A method for the detection of PrPSc in formalin-fixed paraffin-embedded tissue by ELISA has been developed and described by ARS scientists in Ames, Iowa. Methods for diagnosis of TSEs in cattle, sheep and cervids have traditionally depended on the availability of both frozen fresh and formalin-fixed tissues. However, in many diagnostic sample submissions only formalin-fixed samples have been available for TSE diagnosis, a situation that previously precluded analysis by rapid diagnostic procedures such as ELISA. This work describes a method suitable for extraction of the PrPSc from formalin-fixed paraffin-embedded tissue for detection by ELISA. This represents a significant advancement for diagnostic laboratories and provides a rapid alternative method for TSE detection beyond immunohistochemistry (IHC). (NP 103, C7, P.S. 7D, and P.M. 4.2.2, Project #3625-32000-103-00D)

# **NP104**

Accurate mosquito trapping results for precise integrated pest management. Integrated pest management requires accurate information on the number of pests and where they are. This is particularly important for mosquito control because resources are always limited compared to the geographic area requiring treatment. ARS scientists in Gainesville, Florida, compared the number of female mosquitoes captured by suction traps, portable light traps (with carbon dioxide), and landing on a human subject. The results showed that mosquito density from light trap samples was underestimated by 43 to 97 percent and over estimated by 80 to 85 percent, when compared with mosquito landing rates on a human subject. Corresponding values for suction trap samples were 28 to 88 percent and 17 to 87 percent. A statistical algorithm that corrects mechanical trap-based estimates of adult mosquito density to the landing rate of mosquitoes on a human host was constructed, and will provide a better estimate of mosquito density in a local area. This information can be used to help mosquito control professionals determine the best method of control. The algorithm will also enable State and Federal authorities to more accurately compare trapping results from different locations. More accurate estimates of mosquito numbers will help authorities target resources, make accurate risk assessments of disease, and assess with more confidence whether or not invasive species are present. (NP 104, C1 and 3, P.S. 1A and 3F, P.M. 4.2.2, Project No. 6615-32000-045-00D)

Treatment of stable fly larval sites using an organic compliant chemical. Stable flies develop in damp soil with an abundance of incorporated vegetable matter, conditions often associated with feedlots and other cattle operations. The flies feed on the blood of many kinds of animals, including humans, and are considered the principle pest of cattle in the United States, causing \$2 billion per year in loss of yield. Larval control has been difficult because of the widespread distribution of maggots and the difficulty of applying chemicals to them below ground. ARS scientists in Lincoln, Nebraska, developed an encapsuled formulation of catnip oil to control immature stable flies developing in animal wastes. The formulation deters female flies from depositing their eggs and inhibits larval development under field conditions. A single application is effective for five to seven days. This is the first botanical-based product for the control of immature stable flies. The formulation, once registered, will provide an effective stable fly control option for cattle producers. (NP 104, C3, P.S. 3B, P.M. 4.2.2, Project No. 5440-32000-009-00D)

Bed bugs shown to affect human health. Bed bugs are a blood sucking pest that lives in homes, hotels, shelters, vehicles, and businesses. The bugs feed at night causing a variety of reactions from mild irritation to extensive blistering and allergic reaction, though they are not thought to transmit any pathogens. Bed bugs had been controlled effectively in the United States since the 1950s until they became much more numerous starting in 2002. ARS scientists in Beltsville, Maryland, in collaboration with the University of Mississippi Medical Center and Harvard Medical School, showed that bed bug bites can cause a very severe, localized inflammation of blood vessels. Moreover, the type of inflammation that occurs has the potential to affect major body organs. These data show that bed bugs are not only an annoyance, but also a health threat. The impact of this research may be greater involvement to control the national bed bug problem. (NP 104, C1, P.S. 1D, P.M. 4.2.1, Project No. 1245-32000-007-00D)

Novel and effective vaccine for cattle against the cattle fever tick. The southern half of the United States used to be infested with two species of the one host tick that transmitted bovine babesiosis to cattle. The disease is often fatal in adult cattle and is one of the infections that prevent export of live animals. The ticks are abundant in Mexico and elsewhere in the world, so APHIS actively fights re-introduction by requiring special treatment of cattle from areas where the ticks still live, and by maintaining a quarantine zone in southern Texas between Mexico and the United States. Anti-tick vaccines are an attractive idea for control, both to reduce the amount of pesticide required in eradication programs and to manage tick populations overseas where the ticks continue to be a problem. A vaccine based on a tick gut protein, Bm86, has been available in some countries for over 10 years. Recent evaluations performed by ARS in Mission, Texas, in cooperation with APHIS, showed that the Bm86 vaccine is highly effective against one of the species of cattle fever ticks and ineffective against the other. Genomic studies and bioinformatics of the cattle fever tick by ARS scientists produced a series of vaccine candidates based on finding protein sequences likely to cause a strong immune response in cattle. The scientists, in collaboration with EMBRAPA scientists in Campo Grande, Brazil, completed trials of the most promising candidate vaccine antigens, one of which was 75 percent effective against the cattle fever tick that was unaffected by Bm86. The mechanism of action of the vaccine was demonstrated by knocking out the target tick gene with RNAi, showing this particular gene was essential for tick survival. This novel antigen was highly expressed in tick nerve tissues. The results indicate that vaccination against cattle ticks has great potential for integration into APHIS' Cattle Fever Tick Eradication Program and for tick management. (NP 104, C3, P.S. 3A, P.M. 4.2.2, Project No. 6205-32000-031-00D)

Transgenic screwworm produces only males. The screwworm fly is a damaging pest of livestock that infests wounds and eats living flesh often killing cattle and other animals. The screwworm fly used to live throughout the southern United States, but was eradicated by the systematic release of sterile male flies that mated with wild female flies, a procedure developed by ARS. Currently, the fly is prevented from reinfesting these areas by continuously releasing sterile males in eastern Panama as a barrier between South America, where the fly still exists, and Central America, where it has been eradicated. Screwworms are produced in a large factory in Panama, supported by the ARS research program. For the past five years, APHIS funded ARS to develop a transgenic strain of screwworm fly that would only produce males, saving rearing, distribution, and sterilization costs. This project required discovery and insertion of a DNA cassette that was female sex linked and lethal when tetracycline was not added to the larval medium. In 2012, ARS scientists in Kerrville, Texas, and Pacora, Panama, worked with a collaborator at North Carolina State University to successfully produce a strain in which 99 percent of females died if tetracycline was not present in the medium. This strain will enable ARS to prove the concept of the value of a transgenic, male only strain, eventually saving APHIS as much as \$5 million per year. (NP 104, C3, P.S. 3E, P.M. 4.2.2, Project No. 6205-32000-035-00D)

A critical enzyme identified in a sand fly species. Sand flies transmit pathogens that cause a variety of diseases in humans, including verruga, kala azar, cutaneous leishmaniasis, and sand fly fever. Cutaneous leishmaniasis was a major problem for the U.S. military in Iraq, and kala azar accounts for hundreds of thousands of childhood deaths in Africa. ARS scientists in Kerrville, Texas, identified, cloned, and sequenced sand fly acetylcholinesterase, an enzyme which is the target for many kinds of effective pesticides. Collaborative research with the University of Florida is identifying compounds that closely target sand fly acetylcholinesterase. Such active ingredients will not only be effective but safe for people and other non-target organisms. These findings offer the opportunity to develop new insecticides for effective sand fly control. (NP 104, C2 and 3, P.S. 2A and 3H, P.M. 4.2.2, Project No. 6205-32000-033-00D)

Practical control of sand flies for the military. Old world sand flies are responsible for the transmission of leishmania to humans in areas where U.S. military personnel are currently deployed. ARS researchers in Gainesville, Florida, successfully conducted the first study examining the efficacy of ultra-low volume pesticide applications on Old World sand fly species in leishmaniasis-endemic regions in a natural setting in Africa. These findings indicate that by combining ultra-low volume treatments of natural sand fly populations with treatment of camouflage netting, permethrin-treated clothing, and the use of DEET could substantially minimize human-sand fly contact and decrease transmission of leishmania to deployed troops. The outcome of this research could lead to disease risk reduction for U.S. military personnel. (NP 104, C2, P.S. 2A, P.M. 4.2.2, Project No. 6615-32000-045-00D)

<u>Supergene Gp-9 associated with multiple mating and male reproductive success in fire ants.</u> Understanding fire ant male reproductive success and fitness are important components of research aimed at suppressing fire ant

populations, yet data on this important topic are virtually nonexistent. ARS researchers in Gainesville, Florida, determined that some fire ant queens mate with more than one male and that whether a queen mates with more than a single male is determined almost entirely by male genotype. Investigation of the physiological basis for the inability of some males to discourage a second mating revealed that male sperm count also is linked to male genotype, suggesting fire ant queens remain receptive to mating if their first partner does not provide a sufficient quantity of sperm. Understanding the importance of the male genotype in fire ant colony structure is an important basic discovery that uncovers a new pathway for disruption of this invasive species. (NP 104, C5, P.S.5A, P.M. 4.2.1, Project No. 6615-32000-044-00D)

Progress in biological control of fire ants. Imported fire ants are unusually abundant in the United States, probably because they have escaped their natural enemies left behind in South America. ARS researchers in Gainesville, Florida, are evaluating the release of a new phorid decapitating fly, *Pseudacteon cultellatus*, near Miami and Gainesville, where it is beginning to expand out of the release area. This new species of fly specializes on attacking the smallest sizes of fire ant workers, which are most abundant in multiple-queen fire ant colonies. This preference is especially important because multiple-queen fire ant populations average 2 to 3 times the densities of regular single-queen fire ant populations and are therefore a substantially greater pest of homes, agriculture, and the environment. Another species of phorid decapitating fly, *Pseudacteon obtusus*, preferentially attacks larger fire ant workers. This phorid fly was shown to multiply well even in the presence of other species of decapitating flies. This is significant because elimination of the larger worker ants will have a greater negative effect on the colony. (NP 104, C5, P.S. 5A, P.M. 4.2.2, Project No. 6435-32000-012-00D)

Genomics and biochemistry of termites. The threat of the Formosan subterranean termite to the southeastern United States resulted in a robust research program at ARS on this pest in 1997. Through basic and applied research, cooperation with academic institutions, and a demonstration project, the program successfully solved the problem through areawide integrated pest management and literally saved the French Quarter of New Orleans from destruction. Scientific accomplishments continued through the last year of ARS' termite research, with particularly significant contributions on genomics and biochemistry. ARS scientists in New Orleans, Louisiana performed a project focused on sequencing the Formosan subterranean termite genome and its comparison to the native subterranean termite genome. This work was performed in collaboration with researchers at the J. Craig Venter Institute and Purdue University. The project completed 18x coverage of the 926 mb genome using Illumina Nextgen sequencing. In addition, ARS scientists identified and characterized a novel endogenous endo-ß-1,4-glucanase (named CfEG5) in the Formosan Subterranean termite. Eleven of 15 genes belonging to the metabolic mevalonate pathway were identified. This pathway is responsible for the production of the morphogenic hormone, juvenile hormone, which controls caste differentiation. Further progress was made in understanding caste differentiation by identifying differences in the important musculo-neural protein, myosin, between worker and soldier termites. Basic studies of the biochemistry of carbohydrate metabolism led to discovery of an inhibitor that has promise as a safe pesticide. These basic and applied scientific accomplishments form a foundation for the next work on termites performed outside ARS. (NP 104, C4, P.S. 4A, P.M. 4.2.1, Project No. 6435-32000-012-00D)

Operational research in support of APHIS' Cattle Fever Tick Eradication Program. The Cattle Fever Tick Eradication Program depends heavily on dipping cattle in solutions of the organophosphate pesticide, coumaphos. An alternative developed in cooperation between ARS and APHIS involves injection of long-acting doramectin. Although injections of doramectin to eradicate cattle fever ticks require half the number of treatments as standard coumaphos dips and significantly reduces costs of regulatory treatments to ranchers, there is concern that repeated injections at 25- to 28-day intervals could eventually reduce efficacy of treatments. In a study at Edinburg, Texas, cattle were repeatedly injected at 28-day intervals throughout the year, with blood serum concentration used as a predictor of the probability of female cattle fever ticks being able to survive and reproduce by successfully feeding to repletion between treatments. Of the two dosages that were tested, the higher dose had a 100 percent kill rate, and the blood serum concentration never dropped below this level between treatments. Thus, at this dosage it would be impossible for ticks to reach full engorgement between consecutive treatments. Results of this study demonstrated that the trial policy, instituted by APHIS Cattle Fever Tick Eradication Program, of repeatedly treating cattle with doramectin injections at 25- to 28-day intervals for eliminating cattle fever ticks would produce little or no risk of any viable ticks developing to repletion and re-infesting the field between treatment applications. (NP 104, C3, P.S. 3A, P.M. 4.2.2, Project No. 6205-32000-034-00D)

Mosquito variation across the nation. The United States is host to over 150 species of mosquitoes and each region has its own group of problem species. A few species occur across the country and are very important as vectors and

pests. Two of those species are *Culex tarsalis*, a principle vector of West Nile virus in the western United States, and *Aedes vexans*, a severe pest and occasional virus vector across the entire country. In spite of the importance of these species, their genetic population structure has never been thoroughly examined. ARS scientists in Manhattan, Kansas, have initiated a project to collect many different populations of these species and use the most recent genetic methods to determine population genetic structure. Those methods examine the entire genome of individual specimens to make a thorough comparison of the degree of differences across the continent. More than 86 entities (individuals or agencies) collected 454 unique populations making this one of the largest coordinated collections of disease vector mosquitoes in North America. These mosquitoes will be used to determine the differences between populations of the two species with implications for how to control them and which populations are most likely to transmit pathogens. (NP 104, C1 and 3, P.S. 1A and 3F, P.M 4.2.1, Project No. 5430-32000-003-00D)

Control of mosquito larvae with polyoxyethylene tridecyl ether. Surfactants have been used in soft bodied arthropod control for years, but little is known about the mechanism. ARS scientists in Stoneville, Mississippi, found that the combination of both the surfactant chemistry and its specific hydrophilic–lipophilic balance number can create the best insecticidal activity against mosquito larvae and pupae. A chemical was found that is highly lethal to mosquito larvae and pupae. Oils are the only products available that kill pupae, but oils are difficult to use and sometimes damaging to plants. A soluable, pupicidal surfactant could prevent emergence of pupae and kill larvae in one step. This product may also be a valuable adjuvant for other pesticides, improving penetration of the cuticle. (NP 104, C1 and 2 and 3, P.S. 1A and 2A and 3A, P.M. 4.2.2, Project No. 6402-22320-008-00D)

Crop Protection (ARS Goal 4) (303, 304)

Select Examples of Recent Progress:

## NP303

<u>Sudden oak death fungus soil remediation</u>. *Phytophthora ramorum* causes sudden oak death and also seriously impacts the commercial nursery industry due to losses resulting from quarantine issues. The nursery industry badly needs new methods to control *P. ramorum* so that infested nurseries can be removed from quarantine status and resume normal production. ARS researchers in Fort Detrick, Maryland, demonstrated for the first time in a nursery setting that the beneficial biocontrol fungus *Trichoderma asperellum* grown on wheat bran and raked into nursery test plot soil can reduce *P. ramorum* soil populations to non-detectable levels after six weeks. California regulatory agents confirmed these results at a commercial nursery, and the nursery was lifted from quarantine status. The new method will have wide applicability in reducing losses to the nursery industry due to *P. ramorum*. Technology transfer is underway to facilitate development of a commercial formulation of the biocontrol fungus. (NP303; C2; PS2A and 2C; PM 4.2.4; Project #1920-22000-036-00D)

<u>Diagnostic test for the new boxwood blight pathogen</u>. The recent rapid emergence and spread of boxwood blight disease in the United States places the nursery and landscape industry at substantial risk. Boxwood is a high value ornamental nursery crop valued at \$103 million annually. Early and rapid detection of *Calonectria pseudonaviculata* in plants and soil is needed to prevent the spread of this emergent disease which threatens the health and production of U.S. boxwood. ARS scientists in Beltsville, Maryland, developed a DNA-based boxwood blight diagnostic assay capable of detecting the presence of the causal agent of boxwood blight disease. This diagnostic test will be used by plant scientists to halt the spread of boxwood blight and to develop control measures for the disease. (NP 303; C1; PS1; PM 4.2.4; Project # 1245-22000-279-00D)

Extensive cereal disease evaluation protects U.S. wheat and barley from stripe rust losses. Cereal rust expertise provided by ARS researchers in Pullman, Washington, was applied in 2012 to protect the wheat and barley crop from new, emerging strains of the stripe rust fungus. During the 2012 growing season, ARS scientists evaluated more than 18,000 wheat and 5,000 barley lines for resistance to stripe rust in the field, and hundreds were also tested in the greenhouse with cultured stripe rust strains. This enabled U.S. wheat and barley breeders to select lines for advancing new varieties with resistance to new stripe rust strains. The results of the extensive evaluation combined with molecular marker analysis in 2012 resulted in the advancement and release of more than 10 new wheat and barley varieties with increased stripe rust protection. (NP 303; C3; PS3A; PM 4.2.4; Project # 5348-22000-014-00D (ending) and 5348-22000-015-00D (new))

High yielding soybean with resistance to multiple cyst nematode populations. In the United States, nearly \$1 billion are lost in annual soybean production due to a tiny root parasite, soybean cyst nematode. Cultivars with genetically controlled resistance would reduce these losses. ARS researchers in Jackson, Tennessee, in cooperation with the Tennessee Agricultural Experiment Station, released soybean germplasm line JTN-5203 with resistance to multiple pathogens endemic to the mid-southern United States, and with high yield potential. Traditional breeding methods were combined with modern marker assisted biotechnology techniques for rapid advancements. Soybean JTN-5203 will be highly useful as parent material in breeding programs for providing more durable resistance, especially to soybean cyst nematode, while maintaining very high yield potential in development of new cultivars. The soybean line can also be grown directly as an excellent conventional soybean cultivar in the mid-southern United States. (NP 303; C3; PS3A; PM 4.2.3; Project # 6402-21220-009-00D)

New information developed to enhance biological control of aflatoxin contamination in corn. Infection of corn by some strains of *Aspergillus flavus*, and subsequent contamination with the mycotoxin aflatoxin, results in costs of \$923 million (U.N. Food and Agriculture Organization) and can cause illness in livestock and humans. ARS researchers have developed an effective approach to reduce aflatoxin contamination in corn thought biological control using non-aflatoxin-producing strains of *A. flavus*. In 2012, ARS researchers in Stoneville, Mississippi, completed the genome sequencing of three benign strains of *A. flavus*, which will enable the future comparison of the DNA genome sequence with that of the toxigenic strain. These researchers also assessed two novel formulations for applying the non-toxigenic strains via solid application with bioplastic granules and water dispersible granule formulation, and were granted a U.S. patent on "Granular bioplastic biocontrol composition." (NP 303; C2; PS2C; PM 4.2.4; Project # 6402-42000-005-00D)

Optimizing disease management strategies for HLB (citrus greening) in Florida. Raising a productive citrus crop in Florida, where citrus greening is endemic, poses a challenge to citrus growers. An epidemiological model to predict the spatial and temporal dynamics of citrus Huanglongbing (HLB) from infected areas of South Floridawas finalized and validated. A Web-based version of the model, which can be used to test various disease control strategies, has also been developed for grower use. For example, the model output suggests that controlling secondary infections by diseased tree removal and insecticide applications, plus controlling primary infection from new insect immigrations through area-wide control strategies, can reduce disease increase to a manageable 2 to 5 percent increase per year, which appears to be economically sustainable. (NP 303; C2; PS2A, 2B and 2C; PM 4.2.4; Project # 6618-22000-034-00D)

Evaluation of beneficial yeasts to manage fire blight. Yeasts are being evaluated for use against fire blight of apple and pear. The fire blight bacteria are adapted to the flower nectar cup; the point of infection. The yeast serve as competing organisms that primarily suppress the fire blight bacteria on flower stigmas, which is the main access point for cells to invade the flower tissues. ARS researchers in Wenatchee, Washington, demonstrated in orchard trials that the yeast strain *Cryptococcus infirmo-miniatus* CIMyy6, employed for controlling postharvest fungal disease of fruit, also reduces fire blight in apple trees. The potential inclusion of yeasts in fire blight management has received little attention by researchers until recent years. The consideration of yeasts already approved or developed commercially will accelerate their availability for improving biological control of this serious disease. (NP 303; C3; PS3B; PM 4.2.4; Project # 5350-22000-018-00D)

Preplant IPM strategy for managing root-knot nematode in peach. Peach growers in the Southeast often find it an economic hardship to apply fumigants to orchard sites. Finding a nonchemical alternative to preplant chemical control of nematode pests is warranted. ARS researchers in Byron, Georgia, and Beltsville, Maryland, evaluated a tall fescue grass cultivar as a preplant rotation for suppressing the Southern root-knot nematode. Trees planted after a 1-year or 2-year tall fescue grass cover crop and planted in fumigated soil are significantly larger than trees in unfumigated soil. This work provides the essential baseline data needed to develop a nonchemical preplant nematode control recommendation that now appears in the 2012 Southeastern Peach, Nectarine, and Plum Pest Management and Culture Guide. (NP 303; C4; PS4A and 4B; PM 4.2.3; Project # 6606-22000-013-00D)

<u>Soybean dwarf virus</u> is limited by the aphid vector in the United States. Soybean dwarf virus, which significantly affects soybean crops in Japan and other Asian countries, has been identified in multiple locations within the United States. Outbreaks of *Soybean dwarf virus* in the United States have always been limited, perhaps because the primary aphid colonizing soybean (*Aphis glycines*) is reported to be a poor vector of the virus. An experimental analysis conducted by ARS researchers in Frederick, Maryland, demonstrated that specific mutations must occur in

U.S. isolates to enable soybean outbreaks, and these mutations tend to prevent effective transmission of the *Soybean dwarf virus*. This work demonstrates that the virus outbreaks in the United States would require adaptation of the virus to the vector, and that risks associated with *Soybean dwarf virus* for U.S. soybean production are limited. (NP 303; C3; PS3B; PM 4.2.4; Project No. 1920-22000-034-00D)

Soybean mosaic virus genome regions required for aphid and seed transmission. Soybean mosaic virus is transmitted by aphids and through seed, and can cause yield reductions as high as 35 percent in soybean. Infected seeds serve as the primary source of inoculum for the virus in North America and secondary spread occurs by aphids. ARS researchers in Urbana, Illinois, and scientists at the University of Illinois studied the role of virus-encoded proteins in aphid and seed transmission, severity of foliar symptoms, and induction of seed coat mottling. Two virus proteins previously associated with aphid transmission were shown to also be required for efficient seed transmission, and this discovery suggests that interactions of the two proteins are important for multiple functions in the virus life cycle. In addition, two other regions within the Soybean mosaic virus genome were shown to affect transmission through seed and the presence of seed-coat mottling in virus-infected seeds. The information will be useful to pathologists and virologists for development of novel methods to disrupt disease. (NP 303; C2; PS2B; PM 4.2.4; Project # 3611-22000-019-00D)

# **NP304**

Postharvest irradiation treatment controls light brown apple moth. Since the invasive, light brown apple moth was found in California in 2007, several countries have imposed trade restrictions on some of its host fruits and vegetables. ARS researchers in Hilo, Hawaii, found a radiation dose that resulted in zero tolerance (100 percent control) at the most tolerant light brown apple moth stage. Some countries require zero tolerance to access their markets. This information will facilitate the trade of commodities that are hosts of light brown apple moth. [NP304; C 4, PS 4A; PM 4.2.5; Project # 5320-43000-016-00D]

Cold treatment stops coffee berry borer. Green coffee, which is shipped around the world for custom blending and roasting carries the risk of spreading coffee berry borer. ARS scientists in Hilo, Hawaii, tested the freezing tolerance of over 15,000 coffee berry borer insects at three different temperatures and determined the temperature and time at which they could control 100 percent of all life stages. Hawaii State regulators are using this information to implement a freezing treatment protocol that enables coffee growers in an infested area to ship green coffee to other islands without the need for methyl bromide fumigation. [NP304; C 4, PS 4A; PM 4.2.5; Project # 5320-43000-016-00D]

New lures for critical pests. The spotted wing drosophila is an invasive pest of soft fruits, and the brown marmorated stink bug is a pest of many fruits, vegetables, and field crops. Both originated in Asia and are spreading throughout North America. ARS researchers in Wapato, Washington, working with ARS colleagues in Poplarville, Mississippi, and Oregon State Department of Agriculture scientists, isolated and identified a set of chemicals from the odors of wine and vinegar that can be used as a lure for the spotted wing drosophila. ARS researchers in Beltsville, Maryland, and Kearneysville, West Virginia, discovered a male produced pheromone that causes brown marmorated stink bug nymphs and adults to aggregate. Both lures are being combined with traps and will be used to monitor pest populations for treatment and, perhaps, to control the insects via trapping or insecticidal baits. A provisional patent has been filed for the stink bug lure. (NP304; C2, PS 2A/2B; PM 4.2.4; Project #s. 5352-22430-001-00D, 1245-22000-272-00D)

Improvement of the sterile insect technique in fruit flies. Fruit flies of all kinds are a major pest of fruit orchards and a major trade issue. ARS scientists in Tifton, Georgia, have improved the efficacy of the sterile insect technique used to control tephritid fruit flies. They incorporated the juvenile hormone analog methoprene (which coordinates sexual signaling and reproductive development) and a protein supplement into the diets of adult sterile male flies. Flies fed the protein supplement became sexually mature four to seven days earlier and thus mated earlier. The males also attracted more wild mates, thus increasing the mating frequency. The International Atomic Energy Agency and the Food and Agricultural Organization of the United Nations are including the technology in a coordinated research program to improve the efficacy of the sterile insect technique. The technology is now used in Mexico to improve the reproductive performance of sterile Mexican fruit flies that have been released to control invasive populations. (NP304; C 2,PS 2B; PM 4.2.4; Project # 6615-22000-027-00D)

New methods using fungus to kill insect pests. Root weevils, soil grubs, rootworms, wireworms, fruit flies, and root maggots are insect pests that affect a wide variety of agricultural crops, landscape plants, and turf. ARS researchers in Peoria, Illinois, developed new methods to grow and commercially produce a bioinsecticidal fungus to kill these pests. The fungus, *Metarhizium*, also kills lesser meal worm larvae and adults, which are pests in commercial poultry operations. Koppert BV has licensed the technology from ARS through a patent. ARS researchers also developed dried granular formulations of *Metarhizium* which will give homeowners, farmers, and land managers an effective, nonchemical way to control numerous soil dwelling insect pests. (NP304; C 2, PS 2B; PM 4.2.3; Project # 3620-22410-014-00D)

Stabilization of beneficial traits enhances effectiveness of biological control. Biological control (the use of predators, parasitoids, or pathogens in pest suppression) provides a safe alternative to the use of chemical insecticides. However, during mass production biological control agents can lose some beneficial traits, such as virulence and reproductive capacity which can make the agents less effective in pest suppression. ARS researchers in Byron, Georgia, and colleagues at Brigham Young and Rutgers universities, discovered that beneficial trait loss can be prevented by selecting inbred lines. Inbred line technology has been adopted by three commercial companies that produce insect killing nematodes. (NP304; C2, PS 2A/2B; PM 4.2.3; Project # 6606-22000-022-00D)

Plant growth bioregulators found to protect pecan foliage from black pecan aphids. Black pecan aphid causes chlorosis (loss of photosynthetic chlorophyll pigment) through its feeding. ARS researchers in Byron, Georgia, found that treating pecan foliage with certain plant growth bioregulators improve canopy health by lessening chlorophyll degradation by the aphid. Applying a plant growth bioregulator such as gibberellic acid also increases season-long photosynthesis by encouraging longer foliage retainment into the autumn. Return bloom is not affected and there appears to be little or no negative effect on beneficial insects. This new management tool is being adopted by growers. Use of this bioregulator is also applicable to other crops with pests that elicit leaf chlorosis. (NP304; C2, PS 2B; PM 4.2.3; Project # 6606-22000-022-00D)

New genetic modifications yield "sexing strains" in fruit flies. Mass release of sterile, male fruit flies is used to control the insects, but sterile females released along with males do not contribute to fruit fly control and may distract the sterile males, thereby lowering control rates. Conditional-lethality, in which an insect's offspring dies when certain environmental conditions prevail, is a means of eliminating females early in their embryonic development. Females are not released, and rearing costs are reduced. ARS scientists in Gainesville, Florida, developed a conditional-lethal strain of the Caribbean fruit fly through genetic modification; females of this strain only survive when provided an antibiotic-supplemented (tetracycline) diet, so only male progeny are produced on an antibiotic-free diet. The genetic constructs involved will serve to improve the efficacy of control programs that protect U.S. agriculture from fruit flies and other potentially invasive pests. (NP304; C 2, PS 2B; PM 4.2.3; Project # 6615-22000-025-00D)

<u>Disrupting insect diapause to control pest insects</u>. A critical life function of numerous pest insects is the dormant state known as diapause, which allows insects to survive winters and other adverse conditions. Entering and exiting diapause is hormonally controlled by an insect's neuropeptides. ARS scientists in College Station, Texas, in collaboration with scientists at Ohio State University, developed stable versions of neuropeptides of the "Diapause Hormone" that are much more active than the insect's neuropeptides. Unlike the native neuropeptide, two of these novel compounds also prevent the entry into pupal diapause or block its termination when administered at the preceding larval stage of the corn earworm, killing the insect. This discovery will be used to develop a novel, practical, and environmentally friendly strategy to control pest insects by disrupting diapause. (NP304; C2, PS 2A; PM 4.2.3; Project # 6202-22000-029-00D)

Localization of bacterial and fungal pathogens within the southern green stink bug. Southern green stink bugs acquire several opportunistic pathogens from environmental sources as they feed. Some of these pathogens are transmitted to cotton bolls, resulting in boll rot, and subsequent yield losses. ARS scientists in College Station, Texas, identified the pathogens transmitted to cotton bolls by the Southern green stink bug that are responsible for boll rot. They determined that only two of the many pathogens found in the mouthparts, alimentary canal, or head of the stink bug, are actually transmitted into cotton bolls upon feeding. This work provides a much better understanding of the role of the Southern green stink bugs in transmitting pathogens and will be used to develop control measures to improve U.S. cotton yields. (NP304; C2, PS 2A; PM 4.2.3; Project # 6202-22000-029-00D)

Human Nutrition (ARS Goal 5) (107)

**Select Examples of Recent Progress:** 

## **NP107**

New modeling study suggests lower vitamin E requirement. Ninety-three percent of the American population does not meet the current dietary recommendation for vitamin E. However, there is little if any evidence that deficiency of this vitamin exists in the U.S., suggesting the current requirement may be set too high. An ARS scientist in Beltsville, Maryland, in collaboration with scientists at the University of California, Davis, modeled and quantified the kinetics, bioavailability, and metabolism of alpha tocopherol in healthy adults by measuring tiny doses of the radioactively labeled vitamin excreted in urine or feces over 21 days and amounts in blood over 70 days. The new data suggest the true vitamin E requirement is one third of that set in 2000 by the Institute of Medicine and could form the basis for a revision of that recommendation. Lowering the vitamin E requirement would help the Food and Nutrition Service, which administers the School Lunch Program and is required to provide one third of the Vitamin E daily requirement, address the issue of what is actually needed. (NP 107, C 2, P.S. 2B, P.M. 5.2.2, Project #1235-51530-009-00D)

First animal model to study age-related macular degeneration. Age-related macular degeneration (AMD) is the leading cause of blindness among older adults in the U.S. and there are no known means to delay the onset of the disease. ARS funded researchers in Boston, Massachusetts, developed the first animal model of AMD in a strain of mice that shows lesions similar to those in humans. Use of the model will enable study on the basic cellular and molecular mechanisms involved and will enable testing of dietary changes that could reduce the prevalence of AMD in a few months rather than the standard five years it takes in humans. The model has already corroborated the observation that diets leading to high blood glucose levels in humans are associated with greater risk for AMD. (NP 107, C 4, P.S. 4.A, P.M. 5.2.2, Project #1950-51000-075-00D)

<u>Calories available from almonds are lower than those listed on the food label</u>. Because nuts are high fat foods, their calorie content is high and, when eaten in large quantities, might be expected to lead to weight gain. However, observational studies of people eating the most nuts repeatedly find their body weight is lower than average. ARS scientists in Beltsville, Maryland, fed volunteers three doses of whole almonds in a highly controlled diet for 18 days and energy intake and excretion were carefully measured. Almonds provided one third fewer calories than the value on the food label. All nuts are recommended for consumption in the *Dietary Guidelines for Americans* because they provide a number of shortfall nutrients. These data will assist the FDA in updating the calorie content on the food label. (NP 107, C 1, P.S. 1C, P.M. 5.2.2, Project #1235-51530-009-00D)

Raising plasma HDL cholesterol may not always reduce the risk of heart attacks. HDL cholesterol has long been termed "good" cholesterol. Interventions that increased HDL cholesterol levels are expected to reduce the risk of heart attacks. ARS researchers in Boston, Massachusetts, in collaboration with an international consortium, have used genetic technology to thoroughly analyze the strength of the association between high plasma HDL cholesterol and heart attacks by analyzing gene variants in large numbers of cases and controls from 20 studies. The findings clearly demonstrate that some genetic mechanisms that raise plasma HDL cholesterol do not lower the risk of heart attack. These data challenge the long held concept that interventions that raise plasma HDL cholesterol will uniformly translate into reduction of risk. This finding will lead to more individualized dietary recommendations for health. (NP 107, C 2, P.S. 2.A, B &C, P.M. 5.2.2, Project #1950-51000-077-00D)

Dietary vitamin D2 competes with vitamin D3. Vitamin D deficiency leads to loss of bone mineral density and osteoporosis in adults and is common in the U.S. because few foods contain either vitamin D2 (found in some plants and mushrooms) or vitamin D3 (of animal origin). Vitamin D3 is also produced in human skin by sun exposure, but this source is limited by skin pigmentation (thus African Americans are at high risk for deficiency) and lack of sun exposure. Consequently, many Americans seek to improve their vitamin D intake through foods and supplements enriched with the nutrient. ARS scientists in Davis, California, tested the availability of vitamin D2 in healthy humans from mushrooms that had been treated with ultraviolet light to increase their vitamin D2 content. The goal of the study was to determine whether such mushrooms might be a useful source of vitamin D in the American diet. Results of the study showed that vitamin D2 was readily absorbed from mushrooms but that vitamin D3 levels

decreased proportionally to the increase in D2, therefore resulting in no net improvement of vitamin D status. These findings emphasize the complexity of human supplementation and show the importance of testing dietary recommendations for health endpoints. (NP 107, C 2, P.S. 2.B, P.M. 5.2.2, Project #5306-51530-018-00D)

Sodium and potassium consumption in the United States does not meet guidelines. Most Americans exceed the *Dietary Guidelines for Americans* and other Federal recommendations for sodium and potassium intake, according to new data from ARS scientists in Beltsville, Maryland, who collaborated with colleagues from the Centers for Disease Control. Using the National Health and Nutrition Examination Survey (NHANES) data on more than 12,000 adults participating in NHANES from 2003 to 2008, scientists found 91 percent of Americans exceeded the threshold set by the Institute of Medicine for sodium; 99 percent of those at high risk for hypertension consumed more than the sodium level recommended by the *Dietary Guidelines for Americans*. Less than two percent of the sample met the potassium recommendation. The differences between actual and recommended intakes point out that major changes in the types of foods eaten and reformulations of existing products will have to be made if these goals are to be met. Given the large gap between recommendations and consumption, this is further impetus to determine whether increased potassium and calcium offset adverse effects of sodium on blood pressure, as has been suggested in some controlled studies. (NP 107, C 1, P.S. 1A, P.M. 5.2.1, Project #1235-53000-016-00D)

Breast feeding is best for infant development but formula is close. Breast feeding is recommended as the best source of nutrition for infants. There is also controversy about the relative merits of formula made with cow's milk or soy protein. ARS supported scientists in Little Rock, Arkansas, evaluated almost 400 infants four times during their first year of life for development of mental, motor, and language abilities. Breast fed infants scored slightly but significantly higher on some of these measures and at some of the four time points. Both types of formula led to similar scores, which were within the normal range. Although the advantage was very small, these data support the recommendation to breast feed during the first year of life, but they also give reassurance to mothers who are not able to do so because formula feeding results in babies reaching normal developmental milestones. (NP 107, C 2, P.S. 2A, P.M. 5.2.2, Project #6251-51000-007-00D)

Farmed salmon consumption increases plasma levels of omega-3 fatty acids in humans. Increased consumption of omega-3 fats may be associated with a risk reduction for some chronic disease, especially cardiovascular disease, and many Americans are concerned about obtaining sufficient omega-3 fats through the diet. Dietary advice has suggested two servings of oily fish per week, a primary source of omega-3 fats, to reduce the risk of heart disease, but this recommendation was not based on controlled studies in humans. ARS scientists in Grand Forks, North Dakota, conducted a human study and showed that consumption of 4 ounces of farmed Atlantic salmon twice per week significantly increased omega-3 fatty acids in the blood. These data show that eating the recommended amount of oily fish increases omega-3 fat levels in humans, and gives a scientific basis to the dietary advice to eat oily fish. Moreover, the data also show that lower-priced farmed fish are effective for raising omega-3 levels, thus making this dietary advice easier to follow by those in lower economic groups. (NP 107, C 2, P.S. 2.A, P.M. 5.2.2, Project #5450-51000-048-00D)

Low intake of flavonoids increases risk of death from cardiovascular disease. Flavonoids are plant pigments found primarily in fruits, vegetables, nuts, cocoa, and beverages, such as tea and wine that have health benefits in short-term studies. ARS supported scientists in Boston, Massachusetts, collaborated with scientists at the American Cancer Society on a study following 100,000 participants that included dietary assessment and tracking health status. After seven years, about 3,000 study participants had died from cardiovascular disease. Those with higher flavonoid intake were 18 percent less likely to die from cardiovascular disease, and men were 37 percent less likely to die from stroke. Sophisticated statistical analyses of the intake and mortality data revealed a threshold effect rather than a linear dose response, and showed that low intake was responsible for excess risk and higher intake was not beneficial in reducing cardiovascular disease mortality. This addresses one of the fundamental issues in interpreting observational studies on diet and health: namely, is more intake better? This is often the standard interpretation and this study suggests it may be incorrect. Application of this approach should lead to more critical interpretation of data from epidemiological studies and more realistic dietary recommendations for health. (NP 107, C 2, P.S. 2A, P.M. 5.2.2, Project #1950-51530-009-00D)

Breakfast improves memory needed for solving math problems. Nutritionists have long recommended a good breakfast for school-aged children, but there are few definitive data on the benefits of breakfast for school tasks. Memory is critical for learning, but how brain processes that regulate memory function are influenced by morning nutrition in school-aged children has not been determined. ARS scientists in Little Rock, Arkansas, have recorded

brain electrical activity during the performance of mental arithmetic in children who ate breakfast compared with those who skipped this meal. Researchers found that those who ate breakfast were more efficient at solving math problems because irrelevant information did not interfere as much with working memory function. Brain activity measured in children who skipped breakfast showed they required greater mental effort to do the same mathematical processing. These results provide evidence for the beneficial effects of breakfast on learning in school-aged children, and provide support for policies that seek to improve school performance through better nutrition. (NP 107, C 4, P.S. 4.A, P.M. 5.2.3, Project #6251-51000-006-00D)

Environmental Stewardship (ARS Goal 6) (211, 212, 214, 215, 216, 308)

**Select Examples of Recent Progress:** 

## NP211

Improving global agricultural drought monitoring. Monitoring global agricultural drought requires the accurate estimation of root-zone soil moisture availability in agricultural areas. Such estimates are commonly obtained through water balance modeling from ground-based observations of meteorological variables (e.g., rainfall and air temperature), but these meteorological observations are not available over large portions of the globe, and thus international soil moisture predictions for those areas are highly inaccurate. To solve this problem, ARS scientists in Beltsville, Maryland, are exploring a number of different strategies to improve model predictions for these areas, including integrating satellite soil moisture retrievals into existing models and/or the application of new, more complex models. Recent research uses a novel model evaluation strategy—assessing the quality of root-zone soil moisture retrievals obtained from global water balance models and evaluating the impact of both increased model complexity and the assimilation of satellite-based soil moisture retrievals. Results clearly show that the assimilation of satellite-based soil moisture retrievals is a much more effective strategy for enhancing the quality of model-based soil moisture estimates, thereby improving the accuracy of drought detection and monitoring over large geographic areas, particularly where ground-based observations are not available. These findings are highly relevant to a number of Federal agencies (e.g., the National Oceanic and Atmospheric Administration, the U.S. Agency for International Development, and the USDA Foreign Agricultural Service) that are investing significant resources to improve their capability to monitor agricultural drought at the global scale. (NP 211 Component 4, Problem Statement D; Performance Measure 6.1.1, Project No. 1245-13610-028-00D)

Fall planted cover crops can improve water quality in the Upper Mississippi River basin. Fall planted cover crops are a management practice with benefits that include reducing nitrate losses from artificially drained fields. While the practice is widely used in the southern and eastern United States, little is known about the efficacy of the practice in the upper Midwest, which is characterized by long, cold winters and extensive artificial subsurface drainage systems. ARS scientists in Ames, Iowa, used the Root Zone Water Quality Model to predict the impact of a cereal rye cover crop on reducing nitrate losses from drained fields across five Midwestern States. The model estimated that across the region, winter cover crops, planted at main crop maturity in a corn—soybean rotation, reduced nitrogen loss in tile flow by an average of more than 40 percent. The model also indicated that if winter cover crops were planted on the area of the five States draining to the Mississippi River, the potential reduction in nitrate nitrogen losses from drained fields would be about 20 percent of the total nitrate nitrogen load in the Mississippi River. Additionally, the model estimated that the cost of nitrate nitrogen removed by cover crops would be from \$2.08 to \$4.13 per kilogram. The estimated cost is competitive with other management practices that reduce nitrate losses to surface waters. These results are of interest to stakeholders seeking viable ways to reduce hypoxia in the Gulf of Mexico. (NP 211, Component 3, Problem Statement A; Performance Measure 6.1.1, Project No. 3625-13000-009-00D)

Reducing atrazine losses in agricultural landscapes. Atrazine is a popular and economical corn herbicide that occurs in concentrations in drinking water that exceed water quality standards in many supply reservoirs of the upper Midwest. However, the watershed scale impacts of conservation practices aimed at reducing atrazine use have not been quantified, particularly in channelized agricultural headwater streams in central Ohio. ARS scientists in Columbus, Ohio, quantified the conservation practice adoption threshold needed to reduce atrazine concentrations at the watershed scale, and then demonstrated the effectiveness of a USDA National Resources Conservation Service (NRCS) Environmental Quality Incentives Program (EQIP) special incentive addressing atrazine in a drinking water

supply reservoir. These analyses indicate that the implementation of conservation practices in 30 percent or less of a channelized agricultural headwater stream's watershed is not likely to be effective in reducing atrazine concentrations in these streams. Thus, to achieve watershed-scale reductions in atrazine concentrations a participation in conservation practices greater than 30 percent will be required. Understanding the effects of reducing atrazine usage to achieve watershed scale reductions in atrazine concentrations will help in developing management guidelines for agricultural watersheds in the midwestern United States. In a subsequent investigation in a drinking water supply reservoir, NRCS's EQIP program was used to incentivize operators to adopt one of four practices aimed at reducing atrazine concentrations. ARS research demonstrated that the combined effect of adopting these practices was a significant reduction of atrazine in the reservoir. Most importantly, every dollar spent in EQIP practice incentives resulted in a \$2.04 savings for the city of Columbus's downstream water treatment facility, confirming the economic viability of this program. (NP 211, Component 3, Problem Statement A; Performance Measure 6.1.1, Project No. 3604-13000-009-00D)

WinSRFR 4.1 released to the public and the Natural Resources Conservation Service (NRCS). The water use efficiency of gravity (surface) irrigation, the prevalent method of on-farm water application, is typically low but can be substantially improved if systems are designed and/or operated based on hydraulic engineering principles. ARS researchers in Maricopa, Arizona, released Version 4.1 of WinSRFR, a surface irrigation software program that can be used to analyze field evaluation data, estimate field infiltration properties, analyze design alternatives, optimize operations, and conduct simulation studies. The new software features an updated simulation engine that was reprogrammed using a design layout that includes a modern graphical diagnostic and debugging tool. New functionalities include improved simulation capabilities and irrigation modeling. Intended users include university extension agents, farm advisors, irrigation consultants, and NRCS irrigation specialists. (NP 211 Component 1, Problem Statement A, Performance Measure 6.1.1, Project No. 5347-13660-007-00D)

Improved prediction of lateral channel migration. Laterally migrating, meandering streams erode large quantities of fine-grained bank soils, which adversely impacts downstream aquatic resources. Tools have relied on observed migration from historical aerial images to predict future migration patterns, which introduces great uncertainty. With collaborators at the Universities of Illinois and Pittsburgh, ARS scientists in Oxford, Mississippi, co-developed a new computer model that employs the resistance-to-erosion properties of floodplain soils in combination with a meandering channel flow model to predict channel migration rates. The new technology not only improves predicted migration rates but also the pattern of meandering streams. The U.S. Army Corps of Engineers and natural resource agencies in various states have adopted the model to design, locate, and prioritize bank protection and stream restoration works. (NP 211 Component 2, Problem Statement A; Performance Measure 6.1.1, Project No. 6408-13000-023-00D)

Development of a new conservation practice standard. In the young glacial till landscapes of the upper midwestern United States, closed depressions – known locally as potholes – are widely pervasive. Water from surface drainage collects at the lowest spot in the pothole, keeping the area too wet for farming, even when using standard subsurface tile drains in the field. Most potholes that are farmed are drained with subsurface tile, but also have supplemental drainage from a tile riser (a pipe with holes drilled in its sides) that extends vertically above the soil surface. ARS researchers in West Lafayette, Indiana, found that the extent of potholes within a watershed was directly related to concentrations or loads of nutrients lost from that watershed. Research showed that an alternate practice, called a blind inlet, provided greater filtration of surface water from potholes. When drained with a blind inlet compared to a tile riser, watershed-scale phosphorus losses were decreased by approximately 78 percent, and nitrogen losses were decreased by more than 50 percent. Decreased nutrient losses in runoff waters improve water quality, but also save farmers money, increasing their bottom line. In 2012, ARS scientists in West Lafayette worked with the USDA Natural Resources Conservation Service (NRCS) to develop a conservation practice standard, and NRCS in Indiana now offers blind inlets as a cost-sharable practice through their Environmental Quality Incentives Program. (NP 211 Component 3, Problem Statements C; Performance Measure 6.1.1, Project No. 3602-12130-001-00D)

New irrigation practices reduce sediment, nitrogen, and phosphorous losses. Agricultural production must continue to increase to meet the needs of a growing global population with better nutrition. At the same time, it is necessary to improve the environmental footprint of agriculture, particularly losses of sediment, nitrogen, and phosphorus. ARS researchers in St. Paul, Minnesota, showed that these goals can be simultaneously addressed within the U.S. Corn Belt by combining increased landscape water storage with supplemental irrigation. This combination reduces flooding and associated nutrient losses while stabilizing yields and permitting the adoption of alternative cropping practices, like cover crops and living mulches that provide environmental benefits but increase the risk of soil

moisture depletion. Annual precipitation and stream flow have increased substantially over the past 50 years, so there is water available to supply supplemental irrigation if it can be stored during periods of excess. Restoration of wetlands and construction of ponds could provide that storage and have the added benefits of creating new wildlife habitat, serving as a buffer to reduce downstream losses of sediment and nutrients, and providing additional crop biomass for forages or renewable fuels. Producers would benefit from the additional productivity and also from the reduced financial risk associated with irrigation-stabilized yields, while the broader public would enjoy the environmental benefits. (NP 211, Component 2, Problem Statement D; Performance Measure 6.1.1, Project No. 3640-12130-005-00D)

Contaminant transport in karst recharge areas. Karst hydrology, characterized by caves, fissures, and underground streams through limestone, is the most vulnerable groundwater setting for contamination by surface land use activities. ARS researchers in Columbia, Missouri, characterized flow and contaminant transport conducted in two karst recharge area cave systems over a 3-year period. Despite similarities in land use, geology, and weather, water quality in the two cave streams (Devils Icebox and Hunters Cave) was very different. The Devils Icebox recharge area had significantly greater concentrations and total loads of nutrients, sediment, and herbicides than the Hunters Cave recharge area. In the Devils Icebox recharge area, 94 percent of the row crops occurred on high runoff potential soils, as compared to only 57 percent for the Hunters Cave recharge area. Previous research had already demonstrated that these high runoff potential claypan soils are especially prone to the surface transport of contaminants. These new findings led to the development of a stakeholder-led watershed plan for the Bonne Femme watershed, which includes the two cave watersheds, with the primary goal of improving water quality by implementing management practices for protection of karst recharge areas. (NP 211 Component 2, Problem Statement A; Performance Measure 6.1.1, Project No.3622-12310-004-00D)

Canopy temperature as a guide for irrigation scheduling. Canopy temperature has been successfully used for irrigation scheduling in arid environments, but in humid regions the technique is limited by high humidity and intermittent cloud cover. Infrared thermometers are a convenient, non-contact way of measuring crop canopy temperatures that is not affected high humidity or cloud cover. With colleagues from the University of Missouri, ARS scientists in Columbia, Missouri, evaluated an alternate irrigation scheduling method for humid environments using canopy temperatures measured by infrared. Although soybean and cotton yields were not significantly different across a range of irrigation treatments, use of the infrared technique improved irrigation water use efficiency—the additional yield per unit of irrigation water—for corn and cotton. Irrigation is already increasing in humid areas, and many climate change models predict more frequent droughts in these regions in the future, leading to further increases. As demand for water in humid environments increases for irrigation and other purposes, the infrared method shows potential to aid in irrigation scheduling to optimize water management and protect water supplies. (NP 211 Component 1, Problem Statement A; Performance Measure 6.1.1, Project No. 3622-13610-002-00D).

Estimation of biofuel feedstock production potentials from non-forested riparian zones and grass waterways. USDA's *Regional Roadmap to Meeting the Biofuels Goals of the Renewable Fuels Standard by 2022* targets the southeastern United States for delivery of half of the feedstock contributions needed to meet the advanced biofuels goal of 21 billion gallons per year. Using corporate estimates that 34,990 acres must be dedicated to feedstock production within 25 miles of a 36 million gallon per year biofuel conversion facility converting perennial grass feedstocks via cellulosic ethanol production, ARS scientists in Tifton, Georgia used field trial data to estimate that from 6 percent to 38 percent of the needed acreage could be gained from riparian buffers and grassed waterways. The remaining acreage, if taken from agricultural land in the 25-mile radius would be from 3 percent to 18 percent of current agricultural lands. The analysis suggests a potential to produce nearly 530 million gallons of ethanol per year from riparian zones alone around 11 case study cities in the coastal plain of southern Georgia. Another 215 million gallons per year could come from nonprime agricultural lands, the analysis suggested. (NP 211, Component 4, Problem Statement E; Performance Measure 6.1.1, Project No. 6602-13000-023-00D)

## **NP212**

Soil organic matter in grasslands is susceptible to climate change. Soil organic matter contributes substantially to the health of rangeland ecosystems, but how the long-term effects of rising atmospheric carbon dioxide ( $CO_2$ ) and warmer temperatures associated with climate change affect soil organic matter and rangeland plant production are

unknown. ARS scientists in Cheyenne, Wyoming, and Fort Collins, Colorado, in collaboration with the University of Wyoming and the University of Sydney, Australia, subjected portions of a mixed-grass prairie to temperature and CO<sub>2</sub> concentrations projected for the next 50 years. Plant production frequently increased with additional CO<sub>2</sub> and warmer temperatures. However, soil organic matter that is typically resistant to decomposition also increased, thus indicating the potential for a decline in soil quality and health of the rangeland ecosystem. These results suggest that rising CO<sub>2</sub> and temperature may increase plant production in this semiarid rangeland ecosystem, with an associated decrease of soil organic matter and long-term rangeland ecosystem health. The consequences are a potential reduction of yields and reduced economic viability of grazing livestock systems. (NP 212, Component 3, Problem Statement 3A; Performance Measure 6.2.1, Project 5409-11000-005-00D)

Larger rainfall events associated with climate change may reduce semiarid rangeland forage quality. The precipitation accumulation of individual rain events in semi-arid rangelands can be as important as total annual rainfall in controlling the production and quality of forage for livestock. Nitrogen uptake by plants and soil microbes is important for production and quality of forage. Because shifts toward greater rainfall deposition in individual events are predicted with climate change, ARS researchers in Cheyenne, Wyoming, and Fort Collins, Colorado, along with collaborators from Colorado State University, studied how different deposition of rainfall events affected the uptake of soil nutrients by grass roots and by soil microbes in the shortgrass steppe of eastern Colorado. Results showed that deposition in a single rainfall event (0.4 or 0.8 inches) did not affect the timing of nitrogen uptake, but did substantially influence whether plants or microbes were more effective in acquiring nitrogen. Plants were most effective in acquiring nitrogen following smaller depositions, whereas soil microbes were more effective in acquiring nitrogen following larger depositions. The findings indicate that predicted shifts toward larger deposition rainfall events with future climate change may increase forage production but decrease forage quality in semiarid rangelands. The consequences of poor forage quality include reduced livestock weight and quality, and the need to compensate with inputs that raise the cost of producing livestock. (NP 212, Component 3, Problem Statement 3A; Performance Measure 6.2.1, Project 5409-11000-005-00D)

Increased atmospheric CO<sub>2</sub> concentration selectively affects the vigor of non-native plants. For some plants, greater atmospheric CO<sub>2</sub> concentration promotes larger and more vigorous growth, and while this can increase crop yield, it can also foster an undesired, aggressive spread of non-native plants. Lantana and Vinca, including non-native species introduced to the United States as ornamentals and species considered invasive, were grown in enhanced CO<sub>2</sub> concentration by ARS researchers in Auburn, Alabama. Lantana was more responsive to elevated CO<sub>2</sub> than Vinca. Thus, Vinca is less likely to spread aggressively as atmospheric CO<sub>2</sub> increases, whereas Lantana will likely respond to additional atmospheric CO<sub>2</sub> and may require greater effort to control. This study illustrates how non-native species, previously cultivated as non-invasive, have the potential to become invasive as atmospheric CO<sub>2</sub> concentration continues to increase. Identification of species that may create management problems under future CO<sub>2</sub> conditions will help avoid unintended consequences of using non-native species in ornamental plantings. (NP 212, Component 3, Problem Statement 3B; Performance Measure 6.2.1, Project 6420-11120-006-00D)

Deeper application of fertilizer in soil reduces greenhouse gas emissions when used with no- or reduced-tillage. A major greenhouse gas (GHG) source from cropping systems is nitrous oxide (N<sub>2</sub>O) -- a by-product of fertilizer use that has 300 times the GHG warming effect as the same amount of carbon dioxide (CO<sub>2</sub>). No tillage and reduced tillage are used increasingly to enhance soil carbon storage, decrease soil CO<sub>2</sub> emissions, conserve moisture, and reduce erosion, but the effects of both tillage methods on N<sub>2</sub>O emissions have been inconsistent. A global meta-analysis of 239 direct comparisons of GHG emissions from conventional till, no tillage, and reduced tillage was performed by ARS scientists in St. Paul, Minnesota, the University of California-Davis, and Northern Arizona University. In studies where nitrogen fertilizer was initially placed two inches or deeper in the soil, N<sub>2</sub>O emissions tended to be 25 percent lower with no tillage or reduced tillage than with conventional till, especially in humid climates. In contrast, when nitrogen fertilizers were placed closer to the soil surface, N<sub>2</sub>O emissions tended to be greater or no different with no tillage or reduced tillage than with conventional till. These results provide a simple means of optimizing tillage and fertilizer management practices to reduce N<sub>2</sub>O and CO<sub>2</sub> GHG emissions, while maintaining the other benefits of no or reduced tillage. (NP 212, Component 1, Problem Statement 1A; NP 212, Component 2, Problem Statement 2C; Performance Measure 6.2.1, Project 3640-12000-008-00D)

<u>Sensitivity of herbicide volatilization to soil moisture</u>. Efficient herbicide application has been hampered by a lack of understanding of fate and transport mechanisms. Field investigations over the past 14 years by ARS scientists in Beltsville, Maryland; Ames, Iowa; and Riverside, California, have determined that volatilization (vapor loss to the atmosphere) appears to be the most critical pathway for herbicide loss from production fields into neighboring

ecosystems. Herbicide volatilization experiments conducted in Beltsville, Maryland—the longest record of herbicide vapor loss observations worldwide—demonstrated that herbicide volatilization is greatest under warm, wet soil moisture conditions during the day when air near the soil has a tendency to rise as the soil warms. Consequently, all herbicide volatilization models must be revised to account for atmospheric stability and soil moisture conditions. The addition of these terms significantly improves herbicide volatilization models used to help guide herbicide applications on crop fields worldwide. These model improvements have uses in herbicide application practices and demonstrate ways to reduce environmental and economic losses incurred with volatilization. (NP 212, Component 1, Problem Statement 1A; Performance Measure 6.2.1, Project 1245-12660-013-00D; Project 3625-11610-001-00D; Project 5310-12130-009-00D)

Volatilization moves pesticides through the air into South Florida ecosystems. The health of south Florida ecosystems has been declining due to nutrient and pesticide losses from agricultural activities and urban encroachment. The high humidity and temperatures, frequent rainfall and irrigation, soil type, and soil structure enhance the loss of applied pesticides to the atmosphere. ARS researchers in Beltsville, Maryland, and Tifton, Georgia, in collaboration with researchers from the University of Florida, examined the fate of endosulfan, an insecticide previously identified as a potential hazard to aquatic organisms of this region. The scientists discovered that volatilization from the fields, and not drift during application, was by far the most likely emission source to nearby Everglades and Biscayne National Parks. This discovery provides scientists, regulators, extension specialists, and producers with information needed to modify agricultural management practices to protect sensitive ecosystems. (NP 212, Component 1, Problem Statement 1A; Performance Measure 6.2.1, Project 1245-12610-001-00D)

Accurate prediction of pest control and emissions of soil fumigants to the atmosphere. Soil fumigants are an important tool for pest control but are also potential atmospheric pollutants. Improved application methods are needed to ensure crop protection while minimizing fumigant losses to the atmosphere. ARS scientists in Riverside, California developed a predictive model that can be used to simultaneously estimate the efficacy of soilborne pest control and fumigant emissions from field soil. The model provides a new decision support tool for optimizing fumigant application that ensures adequate crop protection while reducing effects on the environment and concomitant economic impact associated with chemical losses. (NP 212, Component 1, Problem Statement 1A; Performance Measure 6.2.1, Project 5310-12130-009-00D)

Maintaining soil sustainability during corn stover harvest. Corn stover (crop residue left on fields following harvest) has been identified as a potential feedstock for biofuels and other bioproducts. However, excessive harvest of corn stover may also rob the soil of nutrients and carbon necessary to maintain healthy soil and sustainable agricultural production systems. Four years of cooperative field research among ARS scientists in Ames, Iowa, the advanced fuels commercial partnership POET-DSM, and Iowa State University showed that to sustain soil carbon concentrations and critical soil functions, corn stover should not be harvested from a field if the average grain yields of the field are less than 175 bushels an acre. This information is a key development of ARS' Renewable Energy Assessment Project (REAP) studies and provides a guideline for enabling the United States to reach goals for increased biofuels and bioproduct production from corn stover, while sustaining soil health for future harvests. (NP 212, Component 4, Problem Statement 4A; NP212 Component 4, Problem Statement 4B; Performance Measure 6.2.1, Project 3625-12000-013-00D)

Narrow-row planting reduces sediment and herbicide losses from cotton production systems. Surface water quality of agricultural lands can be improved by production practices that reduce runoff containing sediment and agrochemicals. ARS scientists in Stoneville, Mississippi, evaluated sediment and herbicide losses from four-to six-leaf cotton planted in narrow and wide rows to determine if a change of row spacing could maintain yields while reducing cultivation impacts on water quality. Planting cotton on flat beds with rows spaced 15 inches apart reduced sediment loss by 38 percent relative to the losses from cotton planted on raised beds spaced 38 inches apart. Planting cotton in narrow rows also reduced herbicide loss relative to that of wide-row systems given similar factors affecting pesticide movement and runoff. Converting from wide- to narrow-row cotton cultivation provides a simple means of reducing sediment losses from fields without a reduction of yields and improves water quality in the area. (NP 212, Component 4, Problem Statement 4A; Performance Measure 6.2.1, Project 6402-12220-004-00D)

Reduced tillage in dryland cropping systems improves soil quality in less than 10 years. Soil quality indexes are useful metrics of the effects of cover crops and tillage practices on soil and water conservation and are thus valuable indicators of the effectiveness of soil management practices needed for a productive and environmentally safe food

supply. ARS scientists in Lubbock, Texas, measured the effects of tillage and adding diverse grains and cover crops to dryland cropping systems on soil quality parameters and water infiltration rates after 8 and 10 years of production. Overall, the indexes showed that soil properties were more sensitive to cropping system differences (adding more biomass to a cotton crop rotation was found to improve soil quality) than tillage differences (conventional versus no tillage), and demonstrated the importance of crop rotations for improving soil quality of dryland cropping systems. Although soil microbial properties were not sensitive to tillage practices, measurements related to soil quality, including organic matter content, soil density, wet and dry aggregate stability, and soil strength showed tillage-induced differences. This study demonstrated that reduced tillage practices can be used to improve soil quality for agricultural production in less than 10 years for dryland cropping systems. (NP 212, Component 4, Problem Statement 4A, 4B; Performance Measure 6.2.1, Project 6208-12000-010-00D)

## **NP214**

Beneficial use of zinc from ground rubber tires. Waste tires are a significant environmental problem, but beneficial uses have been found for this waste. Tires can be used to produce energy or ground rubber to mix with soil to reduce physical compaction in high traffic areas. Tire rubber contains about 1.5 percent zinc because highly purified zinc, which is very low in cadmium and lead, is used in the vulcanization of rubber. ARS scientists in Beltsville, Maryland, tested the use of ground rubber as a zinc fertilizer and as a potting medium component. Additional field testing was undertaken by a cooperator in Iran, where zinc-deficient soils were amended with varying rates of ground rubber or zinc sulfate to increase crop yield and reduce grain cadmium. The tests showed significant reduction in grain cadmium. In another test in Arizona, different rates of zinc were applied as zinc sulfate or ground rubber to field plots of durum wheat cultivars to determine if high zinc fertilizer applications could reduce grain cadmium levels. In this case, where zinc was not deficient, little or no reduction in grain cadmium was observed. This finding may provide the rubber industry additional uses for old rubber tires. (NP 214, Component 4, Problem Statement B, Performance Measure 6.2.1, Project # 1245-12000-040-00D)

Beneficial use of flue gas desulfurization-gypsum in agriculture. The electrical generating industry produces flue gas desulfurization-gypsum during the process of removing sulfur dioxide (SO<sub>2</sub>) from exhaust gases, resulting in large quantities of the product. ARS researchers in Beltsville, Maryland, and other locations have been conducting research to evaluate the beneficial use of flue gas desulfurization-gypsum in agriculture. Ultimately, the adoption of this product for use will depend on "Beneficial Use Designation" at the State level. ARS is cooperating with the U.S. Environmental Protection Agency (EPA) in conducting a full risk evaluation of this use to provide information needed by states to make regulatory decisions about flue gas desulfurization-gypsum use as fertilizer and soil conditioner. Field tests have been conducted to assess the ability of flue gas desulfurization-gypsum to reduce the runoff of phosphate from soils, including soils amended with poultry litter on the surface of hay fields. To date, research has shown that application of flue gas desulfurization-gypsum can significantly reduce runoff phosphorus and arsenic. Other research has not found adverse effects of soils amended with flue gas desulfurization-gypsum compared to unamended soils. These findings will inform EPA as they formulate new rules for the use and disposal of coal combustion byproducts. (NP 214, Component 4, Problem Statement A, Performance Measure 6.2.1, Project # 1245-12000-040-00D)

Injecting liquid manure improves ammonia retention but increases other nitrogen losses. Incorporation of liquid manure into the soil is often recommended to reduce ammonia losses, odor, and nutrient runoff in surface water. However, incorporation with tillage is not compatible with high-residue conservation practices, such as no-till, and is not possible with pasture or perennial forages. Manure injection technologies allow incorporation with limited disruption of the soil surface or plant residue cover. ARS scientists in Beltsville, Maryland conducted a critical review and analysis of the literature and found many publications that show injection of liquid manures can reduce ammonia-nitrogen emissions by 40-90 percent, compared to surface application. However, injection can create anaerobic soil conditions leading to losses of other gaseous forms of nitrogen. Up to half of the nitrogen that is conserved by reducing ammonia emissions can later be lost as N<sub>2</sub> (a chemically inert gas that makes up 78 percent of earth's atmosphere) or as nitrous oxide (a potent greenhouse gas). Improved crop utilization of the nitrogen conserved by reducing ammonia emissions is the most common observation following injection, but this benefit can be minimal. These results provide scientists, nutrient managers, and policy makers with improved estimates of the effects of manure injection on the fate and transport of manure nitrogen that can be used to improve economic return

and minimize adverse affects of manure nitrogen. (NP 214, Component 1, Problem Statement C, Performance Measure 6.2.1, Project #1245-12630-005-00D)

Managing manure to reduce runoff phosphorus losses. Manure application to crop land can contribute to runoff losses of phosphorus which can lead to excessive algae growth in lakes and streams. ARS researchers in Marshfield, Wisconsin, conducted a series of rainfall simulation experiments to assess how the amount of dissolved phosphorus in runoff would be affected by: 1) phosphorus supplementation of dairy heifer diets; 2) manure application method and rate; and 3) the amount of available phosphorus already in the soil. Phosphorus supplementation in the diet resulted in more phosphorus in manure, which led to two to three times more dissolved phosphorus in runoff. Incorporation of manure into the soil reduced runoff phosphorus concentrations by 85 to 90 percent compared to surface application. These results show that large reductions in phosphorus runoff losses can be achieved by avoiding unnecessary dietary phosphorus supplementation; by incorporation of manure; by limiting application rate when applying to crop land; and by avoiding soils with excessive phosphorus. By adopting these practices, farmers can greatly reduce the amount of phosphorus leaving their farms which subsequently will reduce algae growth and eutrophication in surface waters. (NP 214, Component 1, Problem Statement A, Problem Statement C, Performance Measure 6.2.1, Project # 3655-12630-003-00D)

Reducing odor emissions from swine lagoons. Malodorous emissions from swine lagoons are a problem for swine producers and their neighbors. ARS scientists in Clay Center, Nebraska, demonstrated that a product derived from soybean, soybean peroxidase, was effective at reducing odorous chemical emissions from swine manure by 68 to 81 percent. The research has resulted in awarding of additional grant funds from the National Pork Board for further development in commercial swine production facilities. (NP 214, Component 2, Problem Statement D, Performance Measure 6.2.1, Project #5438-41630-001-00D)

Bacterial communities in beef cattle with diets containing corn and wet distillers grains. There is concern that use of wet distiller's grains, a byproduct of the ethanol industry, in cattle feed may create a gastrointestinal environment that allows the growth of bacterial pathogens such as *Escherichia coli* O157:H7 that can impact human health. Since all *E. coli* must compete for resources with other fecal community members, ARS researchers in Lincoln, Nebraska, looked to see what other kinds of bacteria are present with *E. coli* in the cattle gut. Results indicated that *E. coli* experiences fundamentally different microbial communities in animals fed distillers grain compared to animals fed corn. Competition within different communities may reduce *E. coli* O157:H7 in the gut and thus, management of animal diet could be a legitimate practice to reduce the potential for *E. coli* O157:H7 food contamination. (NP 214, Component 2, Problem Statement A, Performance Measure 6.2.1, Project #5440-12630-001-00D)

Subsurface banding of broiler litter is a better method for corn fertilization. The fertilizer value of broiler litter has been recognized by farmers and applied to row crops under both tillage and no-till systems. However, application of poultry litter to the row crops under no-till soil management concentrates litter-derived nutrients at the soil surface, enhances volatilization of nitrogen, and greatly increases nutrient losses in runoff water. Losses of applied nutrients can cost farmers substantial income and degrade air and water quality. ARS scientists in Mississippi State, Mississippi, studied the impacts of broiler litter placement on corn yield and nitrogen utilization under the no-till system and found that subsurface banding of broiler litter substantially increased corn nitrogen use efficiency by 56 percent and grain yield by 16 percent when compared to surface broadcast litter. This indicates that subsurface banding of broiler litter could be an effective management practice for no-till corn and other row crop production and could become a method of choice for applying solid manures, if the technology can be developed and commercialized as a practical option for the producers. (NP 214, Component 1, Problem Statement C, Performance Measure 6.2.1, Project #6406-12630-006-00D)

Use of hydrothermal carbonization products as sorbent of environmental contaminants. Hydrothermal carbonization is an aqueous thermal process that converts organic wastes into a product called hydrochar. ARS scientists in Florence, South Carolina, working with university collaborators, discovered that the hydrochar made from chicken litter and swine manure showed excellent sorption capacity of environmental contaminants such as endocrine disrupting chemicals, herbicides, and polyaromatic hydrocarbons. This high sorption capacity toward these compounds was attributed to the hydrochar's diverse surface chemistry. In addition, it was found that hydrochar surface chemistry can be easily modified with activating compounds to produce more stable hydrochar products. For example, hydrochar activated with citric acid resulted in a more stable hydrochar in soil environments. Formulations and application methods for field uses of hydrochar are in development. (NP 214, Component 1,

Problem Statement C, Component 3, Problem Statement A, Performance Measure 6.2.1., Project #6657-13630-005-00D)

# **NP215**

The enzyme polyphenol oxidase improves silage protein uptake by livestock. When grasses are ensiled, excessive protein degrades so that when the silage is fed to ruminants, the inefficient protein use results in excessive nitrogen excretion in feces and urine. Polyphenol oxidase (PPO), a naturally occurring enzyme that causes browning in fruits like apples, can also decrease protein degradation when it combines with other naturally occurring compounds called phenolics. ARS scientists in Madison, Wisconsin, co-ensiled the high PPO grass orchardgrass with high phenolic tall fescue grass. When the co-ensiled high PPO and phenolic grass mixture was fed to lambs, total protein utilization was improved 10 to 20 percent over control silages, as indicated by decreased nitrogen excretion in urine. On average, at least \$100 million is spent annually to supplement protein lost during ensiling. Incorporating a PPO/phenolic ensiling system would save dairy farmers \$10 million to \$20 million annually in protein supplements and would decrease waste nitrogen in the environment. (NP 215, Component 3, Problem Statement H, Performance Measure 6.3.1, Project No. 3655-21000-046-00D)

Resistance to stem rust mapped to three chromosomes in perennial ryegrass. Genetic improvement of ryegrass for stem rust resistance has been hampered by a lack of information about the diversity and location of resistance genes in this grass. ARS researchers in Corvallis, Oregon, determined that there is an all-or-none resistance gene located on perennial ryegrass chromosome 7 and effective partial resistance genes located on chromosomes 1 and 6 for stem rust. These discoveries lay the groundwork for developing genetic markers to locate stem rust resistance genes. Technology based on these markers will enable breeders to produce rust-resistant varieties of ryegrass, saving growers the expense of purchasing fungicides and reducing the environmental impact of grass seed production. Information about the location of rust resistance genes in grasses will also add to the knowledge base for finding rust resistance genes in cereal grasses such as wheat which is currently threatened with a new strain of stem rust worldwide. (NP 215, Component 4, Problem Statement K, Performance Measure 6.3.1, Project No. 5358-21000-039-00D)

Eastern gamagrass limits excessive weight gain in replacement dairy heifers. Dairy heifer diets that typically contain significant proportions of corn silage or other high energy forages often gain excessive weight that negatively affects their future lactation performance. Consequently, dairy farmers and nutritionists will add straw and other high fiber feeds to rations to cause replacement heifers to limit feed intake and caloric density. ARS researchers in Madison, Wisconsin, showed that gamagrass may provide an alternative to purchasing and processing \$150/ton straw, and that it can easily be ensiled and blended into mixed forage diets. The feed with gamagrass eliminates undesirable feed sorting behaviors by heifers, potentially neutralizes variations in growth performance among pen mates because less aggressive heifers can access the proper diet whenever they reach the feed bunker, and reduces the total caloric density and dry matter intake of alfalfa/corn silage diets. These factors provide heifer producers with an effective alternative management strategy for managing weight gains by replacement dairy heifers, especially when facilities are overcrowded. (NP 215, Component 3, Problem Statement J, Performance Measure 6.3.1, Project No. 3655-21000-047-00D)

Online weather-based recommendations for rangeland vegetation restoration. The weather across most western rangelands is highly variable and often has a great impact on the success or failure of most rangeland restoration efforts. ARS scientists in Boise, Idaho, created a microclimatic index of favorable seedbed conditions and developed a management framework for using historical weather data for planning and managing rangeland restoration. This framework includes analysis of site-specific historical weather data, evaluation and ranking of annual, seasonal, and monthly precipitation and temperature patterns, and assessment of seedbed microclimatic conditions relative to the growth stage of seeded plant materials. Available via the Web (<a href="www.ebipm.org/">www.ebipm.org/</a>) or as a spreadsheet template, implementation of the weather-based restoration planning framework will improve the success of fire rehabilitation and other projects across western rangelands, reduce restoration costs, and provide land managers a mechanism to adapt restoration efforts to fit site-specific weather conditions. (NP 215, Component 1, Problem Statement C, Performance Measure 6.3.1, Project No. 5362-13610-009-00D)

Rangeland Hydrology and Erosion Model tool implemented. ARS scientists in Reno, Nevada, in collaboration with ARS scientists in Boise, Idaho, and Tucson, Arizona, have developed the Rangeland Hydrology and Erosion Model (RHEM) to estimate runoff and erosion rates on non-Federal rangelands in the 17 Western States and provide maps of where targeted place-based conservation practices to reduce soil loss would be most cost effective. The spatially unbiased nature of the range land assessment allows rapid determination of regional needs and identification of where conservation programs may be most cost effective in arresting land degradation. This same concept can be utilized to target specific locations for conservation practices to meet specific goals in cost effective ways. RHEM was used to assess benefits of conservation in Texas from place-based conservation to reduce soil erosion and restore ecosystem services for a demonstration watershed. This work is being highlighted in the USDA National Conservation Program report to be delivered to Congress this winter. (NP 215, Component 1, Problem Statement A, C; Performance Measure 6.3.1, Project No. 5370-11220-006-00D)

Prescribed burning and grazing can help restore wildlife habitat. Historically, both fire and grazing have influenced vegetation structure and composition and ecosystem functions of the Great Plains. Land managers of the 20th century focused on reducing the occurrence of fires and prairie dogs to promote livestock production. Subsequent declines in populations of many grassland birds have prompted land managers to consider restoring fire and prairie dogs to produce a more suitable habitat for grassland birds. ARS scientists in Cheyenne, Wyoming, and Fort Collins, Colorado, studied the nesting habitat of a grassland bird (Mountain Plover) in relation to prescribed fire and grazing by cattle, with or without prairie dogs. Prescribed burning and grazing by cattle with prairie dogs are effective strategies to manage for Mountain Plover habitat. These management practices, however, have tradeoffs with regards to beef production associated with traditional grazing management strategies, showcasing the difficulty in managing for both production and conservation goals. (NP 215, Component 1, Problem Statement C; Performance Measure 6.3.1, Project No. 5409-22610-001-00D)

Rodents can enhance restoration efforts in Great Basin environments. Seed harvesting, consumption, and dispersal through caching by seed-eating desert rodents all have profound impacts on specific plant species and on species composition of arid plant communities. ARS scientists in Reno, Nevada, have studied the feasibility of utilizing the seed dispersal services of native animals as a passive restoration strategy. Seed dispersal was successfully tested at a field scale by broadcasting millet as a "diversionary seed" over 2.5-acre plots in areas where rodents typically cache Indian ricegrass seeds, an important native species, in abundance. Under these circumstances, rodents cached and preferentially recovered the diversionary seeds before beginning to consume the less desirable Indian ricegrass seeds. Consequently, more Indian ricegrass seeds were available for emergence as seedlings using this passive restoration scheme. (NP 215, Component 1, Problem Statements A, C & NP 304, Component 3, Problem Statements A, B; Performance Measure 6.3.1 & 2.2.3, Project No. 5370-11220-006-00D)

Glyphosate can control rust disease in herbicide-tolerant alfalfa. Foliar diseases can reduce alfalfa yields by 10 to 50 percent and also reduce forage quality. ARS researchers in St. Paul, Minnesota, found that glyphosate, a widely used herbicide, completely controlled alfalfa rust on glyphosate-tolerant plants inoculated with the fungus 3 days after the herbicide was applied. The level of protection declined with time after application, indicating that control is transitory and that protective treatments inhibit fungal growth. Complete control of rust was obtained when glyphosate was applied up to 10 days after inoculation with rust spores, indicating that the herbicide also has curative activity. Glyphosate treatment increased protection from anthracnose disease and reduced symptom severity from spring black stem and leaf spot disease, as well. These results indicate that as glyphosate is increasingly used to control weeds in herbicide-resistant alfalfa, concurrent reductions in foliar diseases in glyphosate-tolerant alfalfa can be expected to increase herbage yields and forage quality. (NP 215, Component 3, Problem Statement J; Performance Measure 6.3.1, Project No. 3640-12210-001-00D)

## **NP 216**

Cover crops may negatively influence vegetable germination. Cover crop residues often release allelochemicals that can reduce germination and seedling growth of other plant species, which can be part of the mechanism by which they help reduce weed pressure in cropping systems. These chemicals may also inhibit establishment of cash crops because they are not specifically targeted to weedy plants. Sunhemp is a tropical legume with significant potential for use as a cover crop in the southeastern United States. Its residues suppress weeds, but their effects on cash crops are relatively unknown. ARS scientists from Beltsville, Maryland, in collaboration with University of Georgia

scientists, evaluated the effects of sunhemp residues and cereal rye residues on weeds and vegetable crops in greenhouse and growth chamber experiments. In the greenhouse study, dried sunhemp residues and rye residues similarly suppressed germination of lettuce and smooth pigweed (a weed). Sunhemp leaf tissue had a greater effect than did its root tissue. In a second study, water extracts of sunhemp leaves significantly inhibited germination of bell pepper, tomato, and onion, and suppressed turnip and okra germination by 50 percent, while germination of sicklepod, a common weed in southern cropping systems, was not affected by the extracts. In addition, sunhemp leaf extracts inhibited root elongation of lettuce, carrot, smooth pigweed, and ryegrass. These results indicate that sunhemp residues can help reduce weed pressure, but field-scale evaluations are needed to assess subsequent crop germination under actual growing conditions. (NP 216, Component 1, Problem Statement A, Performance Measure 2.2.1, Project No.1245-21660-003-00D)

Assessing food cropping and production patterns in the Northeast with new geodatabases. Development of effective regional food systems holds much potential for improving health, nutrition, and economic well-being. But before progress can be made toward improving the access, affordability, and appropriateness of locally produced food in the Northeast, better tools are needed to analyze and assess current and future food production capacity within the region. ARS researchers from Orono, Maine, in cooperation with a team of university researchers and ARS scientists from Beltsville, Maryland, developed a 13 State collection of geodatabases that brings together available spatial information on cropping systems and crop production, soils, land use and quality, and water resources. These mapping products provide integrated information on past and present farm land extents and productivity and are being used with forecasting models for improving future farm and crop productivity. Through integration of multiple layers of useful data in these geodatabases, this work will be used to help improve the access and affordability of locally produced food for the northeast region. (NP 216, Component1, Problem Statement A, Component 2, Problem Statement A, Performance Measure 2.2.1, Project No.1245-21660-003-00D)

Oat and rye cover crops substantially reduce nitrate losses in drainage water. Much of the nitrate in the Mississippi River comes from land used to produce corn and soybean. Cover crops grown between maturity and planting of these crops are one approach for reducing losses of nitrate. ARS scientists in Ames, Iowa, in a field study showed that a rye winter cover crop reduced the concentration of nitrate in drainage water by 48 percent over five years, while an oat fall cover crop reduced nitrate concentrations only by 26 percent. However, both oat and rye cover crops are viable management options for reducing nitrate losses to the Mississippi River from land used for corn and soybean production. (NP 216, Component 4, Problem Statement D, Project No. 3625-21610-001-00D)

Cover crop biomass production and nitrogen accumulation in high-input, tillage-intensive production systems. Growers need reliable information on cover crop performance over years with different winter conditions. ARS researchers in Salinas, California, evaluated cover crop biomass production and nitrogen accumulation over eight winters in a rotational study with organic vegetables. Rye and a legume-rye mix rotation produced 25 percent more cover crop biomass than mustard and the legume-rye mix accumulated 35 percent more nitrogen. This research provides growers with critical information to choose the most cost-effective cover crops to maximize organic matter inputs that can help maintain and improve soil quality and vegetable yields with minimal fertilizer inputs, and minimize nitrate leaching into ground and surface waters. (NP 216, Component 2, Problem Statements A, B; Performance Measure 2.2.1, Project No.5305-21620-012-00D)

GPS guided drill operation captures runoff on steep slopes. Contour farming has long been recommended as a means of retaining water on hill slopes and preventing soil erosion. A geospatial positioning system method was developed and validated by ARS scientists in Pendleton, Oregon, to guide a tractor and its seed drill along the elevation contour lines on a hill slope. Results demonstrated that seeding precisely in one pass on the elevation contour of an upper shoulder slope can effectively capture and hold the runoff from a 100 year, 24 hour storm event. Using terrain map information and GPS-based autosteering systems, contour seeding promises to improve soil and water conservation in many tillage systems. The method can be implemented using commercially available mapping software and autosteering equipment designed for tractors and drills. (NP 216, Component 4, Problem Statement A, Performance Measure 2.2.1, Project No.5356-21610-001-00D)

Sheep grazing can help lower agricultural greenhouse gas emissions. Currently, agriculture contributes about 6 percent of the U.S. greenhouse gas emissions. ARS scientists in Sidney, Montana, collaborated with scientists from Montana State University to show that using sheep to control weeds during fallow periods of crop rotations may also reduce greenhouse gas emissions. A 2-year study showed that sheep grazing has little impact on greenhouse gas emissions compared to the herbicide method of weed control under dryland cropping systems while still maintaining

crop yield and quality. Less intensive sheep grazing with reduced nitrogen fertilization rates can be used to mitigate greenhouse gas emissions and sustain crop yields. Consequently, both animal and crop producers may benefit from sheep grazing during fallow as an effective and potentially inexpensive method of weed control that not only can sustain crop yields, but also mitigate greenhouse gas emissions. (NP 216, Component 1, Problem Statement A, Performance Measure 2.2.1, Project No.5436-13210-005-00D)

Integrated crop-livestock systems maintain soil quality. Integrated crop-livestock systems, which combine crops and cattle, benefit farmers agronomically and economically. An unanswered question, however, is how livestock influence soil quality for succeeding crops. ARS scientists in Mandan, North Dakota, compared the soil quality of an integrated winter grazing management system with perennial grass pastures, which are considered the "gold standard" for soil quality. After 9 years of detailed observation, the soil quality of the integrated winter grazing system equaled that of the perennial grass system on these northern Great Plains soils. This new information will benefit farmers in the northern Great Plains by assuring them that they can graze crop residue without a negative impact on soil quality. (NP 216, Component 1, Problem Statement A, Performance Measure 2.2.1, Project No.5445-21660-002-00D)

Powered rolling and crimping device developed for small farms. In small vegetable/organic systems, bigger tractors are not used because they are too expensive, heavy, and large for small planting areas. Instead, 2-wheel walkbehind tractors and small implements are utilized widely, but there were no small rollers/crimpers available to effectively crimp and terminate cover crops without herbicides (as required in organic systems). An effective, low weight roller/crimper compatible with 2-wheel tractors was needed. ARS scientists in Auburn, Alabama, developed a unique powered roller/crimper for self-propelled walk-behind tractors. The development of this patented powered roller/crimper is important for small no-till organic vegetable systems, where commercial herbicides are banned, and traditional rollers are too heavy for limited power 2-wheel tractors and too large for narrow beds typical of smaller farms in the United States and worldwide. (NP 216, Component 2, Problem Statement A, Performance Measure 2.2.1, Project No.6420-12610-004-00D)

# **NP308**

Alternatives to methyl bromide for clean commodities in California. Fumigation for insects is necessary to export many kinds of products, and to maintain the wholesomeness of domestic products. For decades, industry has relied on methyl bromide. The advantages of methyl bromide are that it is cheap, penetrates most packaging and commodities to reach insect pests, and kills all stages of most insects. Finding alternatives to methyl bromide that are both effective and do not harm commodities has been a priority. Experiments conducted by ARS researchers in Parlier, California, demonstrated the insecticidal efficacy of sulfuryl fluoride and phosphine, dispensed by a Horn generator. These fumigants appear to be the primary chemical alternatives to methyl bromide for postharvest disinfestations of perishable and durable commodities in California. In other research, ARS researchers in Parlier, California, in collaboration with Washington State University, have found a successful alternative treatment that does not require fumigation. The scientists found that a low pressure, low temperature treatment kills codling moth in fresh fruit and results in a higher quality product than when the produce is treated with methyl bromide. This research helps to limit the current need for methyl bromide while protecting American agricultural interests. (NP 308, C 2, P.S. 2C, P.M. 4.2.3, Project #s 5302-43000-033-00D and 5302-43000-034-00D)

Emission reduction with totally impermeable film. Tarping fumigated fields with totally impermeable film can significantly reduce emissions, but can also increase fumigant residence time in soil and require extended tarp-covering durations to reduce potential exposure of workers and bystanders to fumigants. In collaboration with university researchers and industry, as well as regulatory agencies, ARS scientists in Parlier, California, conducted a large field study in 2011 and found low emission flux of a mixture of chloropicrin and 1,3-dichloropropene (Pic-Clor 60) throughout a tarp-covering period of 16 days with total emission loss of less than 10 percent, and less than 1 percent at the tarp edges. Emission flux upon tarp-cutting increased, but was substantially lower than emissions when the tarp was cut 5 or 6 days after covering. This study demonstrated the ability of totally impermeable film to significantly reduce fumigant peak flux and total emissions and also documented the need for a longer wait time before tarp-cutting time when using the totally impermeable film. (NP 308, C 1, P.S. 1B, P.M. 4.2.3, Project N# 5302-13220-004-00D)

Management of red flour beetles in rice mills without methyl bromide. The red flour beetle is the most important insect pest infesting rice mills, and management has historically relied on structural fumigation with methyl bromide. Because this use of methyl bromide is being phased out under the Montreal Protocol, there is a critical need to evaluate the efficacy of alternative treatments. ARS scientists in Manhattan, Kansas, and colleagues at Kansas State University determined that the average reduction in capture of beetles in pheromone traps after 25 sulfuryl fluoride fumigations in six mills was 66 percent. Rice mills are not as tightly controlled against the weather as wheat mills. As a result, the beetles follow a strong seasonal pattern both inside and outside the rice mills, with highest numbers during warm months. Another significant aspect of the relatively open nature of rice mills is that outdoor populations of beetles evidently serve as a reservoir for the indoor populations and their numbers tend to go up or down together. As a result, fumigation and control measures are strongly influenced by the weather. These data suggest fundamental difference in red flour beetle populations in rice and wheat mills, so that fumigation of rice mills must be timed according to different criteria. The timing of fumigation in rice mills is critical to maximize the efficacy of alternatives to methyl bromide. (NP 308, C 2, P.S. 2A, P.M. 4.2.3, Project # 5430-43000-028-00D)

Induction of nematode suppressive soil system. The tree fruit producer community needs effective non-chemical strategies for long-term control of plant parasitic nematodes because no nematicides are available for post-plant application. ARS scientists in Wenatchee, Washington, examined pre-plant application of *Brassicaceae* seed meal formulations used in conjunction with a virtually impermeable film for control of apple replant disease and suppression of lesion nematode in two organic orchard systems. Seed meal formulations provided multi-year suppression of the lesion nematode densities in apple roots to levels significantly below the no treatment control. Although soil fumigation significantly suppressed densities of this nematode during the initial growing season, extensive re-infestation of fumigated soil by the nematode was observed during the two growing seasons to densities dramatically higher than the control or seed meal treated soils. Nematode suppression in the seed meal treated soil was associated with significant changes in soil biology, including increased densities of nematode parasites and predators. This research indicates that a biologically viable alternative to chemical nematicides or soil fumigants can provide extended long-term suppression of plant parasitic nematodes in orchard production systems. (NP 308, C 1, P.S. 1A, P.M. 4.2.3, Project # 5350-22000-017-00D)

Oxygen and phosphine as an alternative to methyl bromide. Phosphine is commonly used as a fumigant and it does not degrade the ozone layer as does methyl bromide. The effectiveness of phosphine, whether generated from metallic phosphides or applied in pure form, is not as certain as for methyl bromide. Fumigation of leafy vegetables is a particular challenge because the tissues of the plants are easily damaged by fumigation and must be kept at cool temperatures to assure longevity of the product. ARS scientists in Salinas, California, have discovered that the addition of oxygen to phosphine fumigations greatly increases the effectiveness of this fumigant at low temperatures. Oxygenated phosphine fumigation was demonstrated to significantly reduce treatment time and phytotoxicity and achieve effective control of tolerant insects that cannot be controlled with regular phosphine fumigation. Oxygenated phosphine fumigation has the potential to make significant impact on the fumigation industry and replace the need for methyl bromide in some situations. (NP 308, C 2, P.S. 2C, P.M. 4.2.3, Project # 5305-43000-003-00D)

New grafting techniques for tomatoes. Graft angle has a profound effect on the survival of herbaceous grafted plants before and after planting in open field production. Initial survival increased with angled cuts at 70 degrees rather than 20 or 45 degrees. The greater angle exposes more surface area to allow greater cell to cell contact. Also, the greater the graft angle the greater the force necessary to separate the scion and the rootstock at the graft union. This is an important factor when planting plants in open field production where conditions of wind and rain events can compromise plant survival. The increased survivability using this method will reduce the need for overplanting by as much as 30 percent. (NP 308, C 1, P.S. 1A, P.M. 4.2.3, Project # 6618-22000-035-00D)

Tool to target stored products beetles. Knowing when and where to treat stored products pests is important to limiting the use of fumigation and other pesticide treatments. Accurate surveillance can also increase the effectiveness of integrated pest management by enabling applicators to concentrate efforts where the problem is most severe. ARS scientists in Gainesville, Florida, have developed a new trapping system for stored product beetles. Studies of several species of beetle pests of stored grains showed that all are preferentially attracted to light at a wavelength of 390 nanometers. Light at the optimum wavelength and a design that took advantage of beetles' tendency to orient towards edges were used to create a trap 20 times more effective than the current industry standard. Development of this new trapping system significantly improves the ability to monitor stored product pests even when pests are present at extremely low levels. The system promises to significantly reduce pesticide use

for control of these pests because, instead of whole mill or warehouse fumigation, only specific areas need to be treated. (NP 308, C 2, P.S. 2A, P.M. 4.2.3, Project # 6615-22430-003-00D)

Methyl bromide alternatives for Prunus replant disease. Tree growth and yield data collected from 10 orchard replant trials by ARS scientists in Davis, California, demonstrated that broadcast, strip, and GPS-controlled spot fumigation treatments with 1,3-dichloropropene, chloropicrin, or mixtures of the two fumigants provided equal or superior control of Prunus replant disease, compared to soil fumigation with methyl bromide. Pre-plant spot treatments with steam and pre- and post-plant treatments with various fungicides (all applied to tree planting sites) were ineffective. (NP 308, C 1, P.S. 1A, P.M. 4.2.3, Project # 5306-22000-014-00D)

# Management Initiative 2: Ensure Provision and Permanent Access of Quality Agricultural Information for USDA, the Nation, and the Global Agricultural Community via the National Agricultural Library

The National Agricultural Library (NAL) is the largest and most accessible agricultural research library in the world. It provides services directly to the staff of USDA and to the public, primarily via the NAL Web site, <a href="http://www.nal.usda.gov">http://www.nal.usda.gov</a>. NAL was created with USDA in 1862 and was named in 1962 a national library by Congress, as "the primary agricultural information resource of the United States." NAL is the premier library for collecting, managing, and disseminating agricultural knowledge. The Library is the repository of the Nation's agricultural heritage, the provider of world class information, and the wellspring for generating new fundamental knowledge and advancing scientific discovery. It is a priceless national resource that, through its services, programs, information products, and Web-based tools and technologies, serves anyone who needs agricultural information. The Library's vision is advancing access to global information for agriculture.

# **Selected Examples of Recent Progress:**

VIVO. This is a semantic, Open Source, application developed at Cornell University that enables the networking of scientists. NAL is coordinating and providing the technical support to modify the VIVO application and make it operational at USDA. VIVO lists the USDA scientists' research, grants, patents, publications and more. The application is available within USDA and contains content drawn from the targeted five science agencies: ARS, the Economic Research Service, the National Agricultural Statistics Service, NIFA, and the Forest Service. The next data set to be added is the CRIS extramural activities from NIFA. Public availability is scheduled for the fall of 2012. It is currently accessible at <a href="http://vivo.usda.gov">http://vivo.usda.gov</a>.

Start2Farm clearinghouse database. In partnership with the American Farm Bureau Federation, NAL's Alternative Farming and Rural Information Centers has created Start2Farm a Web-based educational clearinghouse at <a href="https://www.start2farm.gov">www.start2farm.gov</a>. The Web site is designed to assist people new to or with less than ten years experience in farming or ranching. It contains training materials, curriculum, and events calendar. The project is funded through a grant from the NIFA Beginning Farming and Ranching Development Program.

Life Cycle Assessment Digital Commons. Life Cycle Assessment (LCA) is a technique to assess the environmental aspects and potential impacts associated with a product, process, or service. The goal of the LCA Digital Commons Project is to develop a database and tool set intended to provide data for use in LCAs of food, biofuels, and a variety of other bioproducts. The LCA Digital Commons now contains field crop production unit process data. NAL is coordinating with other Federal agencies to link database development efforts, and is working with NIFA to require the uploading of research data as part of its bioenergy grants and other data sets. The total Digital Commons Web site is operational and accessible by the general public at <a href="www.lcacommons.gov">www.lcacommons.gov</a>.

AGRICOLA Index expansion through automatic indexing. NAL has chosen and is implementing automated indexing/text analytics software to produce the AGRICOLA Index of agricultural literature. The AGRICOLA Index is publicly accessible and provides subject access to scholarly articles and information in the agricultural sciences. This application combines semantic analysis, machine learning, and human rules to automatically assign subject terms to journal articles. The software passed a rigorous testing procedure and is now being tuned to increase output.

Collection development policy. NAL's Collection Development Policy that governs how the Library grows and maintains its collection has been revised and updated. This process involved input for ARS, USDA, and the scholarly community. The new policy sets forth the scope, level of collecting intensity, and retention factors for NAL. It guides NAL's activities in serving USDA, the U.S. government as a whole, the scholarly community, and the general public at

www.nal.usda.gov/about/policy/collection\_development/NAL\_Col\_Dev\_Policy\_2012.pdf.

Designates accomplishments used in FY2014 Explanatory Notes